Studies on the Life History and Habits of a Previously Undescribed Species of *Sphaeronema*, a Nematode Parasite of Sweetgum (*Liquidambar styraciflua*)

By

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INTRODUCTION

In 1956 specimens of a previously undescribed cyst nematode were found east of Gainesville, Florida. Mr. Gilbert M. Whitton found the mature females attached to the roots of a sweetgum tree, Liquidambar styraciflua L., growing on the bank of Hatchet Creek approximately 8 miles east of Gainesville. Soil samples from around the roots of the tree contained cysts and unidentified larvae, apparently of the species in question. Dr. J. R. Christie was of the opinion that this nematode was probably a member of the genus Sphaeronema.

Dr. Christie called my attention to this interesting organism and suggested that a study of it would be most worthwhile. Nematologists are uncertain as to the phylogenetic position of the genus, and information concerning this species might prove of value in understanding its relationship to other genera of sedentary plant parasitic nematodes.

We then collected soil and sweetgum roots from the Hatchet Creek location and started cultures of the nematode on sweetgum seedlings growing in infested soil in clay pots. Numerous sweetgum trees along Hatchet Creek and in the Gainesville area were sampled in an attempt to find this nematode in other areas. We found only one specimen as a result of these efforts. Later one mature female was recovered from soil around a sweetgum seedling on the banks of Green Sink on the University of Florida campus. Subsequent sampling in the area failed to produce

more specimens. The number of larvae which developed on seedlings in pots was quite low. Shortly after the material was collected the State Road Department, in a bridge repair project, removed all of the trees and much of the original soil from the collecting site. Further efforts to collect this nematode from this site failed; therefore, the number of nematodes available for this study was of necessity limited.

The work reported here is primarily the results of a study of the identity, development, morphology, physiology, and behavior of this nematode. The species is named for its discoverer.

Experiments were conducted to obtain information, particularly on the life history, which would result in a better understanding of the possible kinship of this species to other sedentary plant parasitic nematodes.

The following is a review of the life cycles of four genera of sedentary plant parasitic nematodes. These are discussed because of the similarity of their life cycles to that of S. whittoni.

SEDENTARY PARASITES

Nematode life cycles are commonly characterized by four molts and five stages. Christie (1941) remarks: "It is customary to regard the first molt as terminating the first and initiating the second larval stage, the second molt as terminating the second and initiating the third larval stage, etc." According to Hyman (1951) four molts in which the entire cuticle, including the lining of the buccal capsule, pharynx, rectum, and vagina, is shed probably occur in all nematodes.

Hatching in the sedentary parasites, as in most nematodes, requires favorable oxygen tension, moisture, and temperature conditions. These requirements vary with the species.

Both male and female larvae of most nematode species are eel-like in shape and resemble adults except for the undeveloped condition of the reproductive system. There are, however, several interesting genera of sedentary plant parasitic nematodes in which the larvae and adult males are eel-shaped and apparently do not feed, but the female larvae become swollen and the adult pear-shaped or spherical. This type of development is typical of species of the genera Rotylenchulus and Tylenchulus. The two genera Meloidogyne and Heterodera are unique in that both male and female larvae increase in body diameter but at the time of the final molt the male undergoes a metamorphosis from which it emerges as a slender eel-shaped adult.

Meloidogyne

Although the species of this genus differ in their host-parasite relationships and physiology, they all have substantially the same life history (Christie, 1959).

Christie and Cobb (1941) confirmed the observations of Nakagura (1930) that the first molt occurs within the egg when they observed the separated cuticle protruding beyond the head of the larva while it was still unhatched. The first molt takes place before the larva has reached maximum length and while it is relatively inactive. When molting specimens were removed from the egg they moved sluggishly but if the larvae moved actively the molt had been completed.

The newly hatched second stage larvae are slender worms 400 μ to 500 μ long. They move about in the soil and will enter almost any plant tissue that is in contact with the soil. Dropkin (1957) found that about 80 per cent of the larvae penetrated tomato roots by the end of the second day of contact with the plant. After that time little or no entry occurred. Most of the infective larvae enter at or near the root tip (Christie, 1936) and after becoming situated within the tissues of the host, the larvae do not move or change their position. The male is a sedentary parasite only during larval development but the female is sedentary throughout her entire life.

Early larval growth consists of increase in width in both sexes. They become oval in shape and are about one half as wide as they are long (Christie, 1959). Second stage larvae of Meloidogyne javanica (Traub, 1885) double their diameter. Growth is more rapid in the next five days,

trebling the cross sectional area during that period (Bird, 1959).

Larvae of M. javanica undergo the second molt approximately fourteen days after entering the host tissue. The third and fourth molts occur in rapid succession during the next three days. During the second molt the stylet tip is shed with the cuticle and the stylet shaft and knobs break down. There is no stylet in either the third or fourth stage larvae.

The males undergo metamorphosis within the sac formed by the second and third molted cuticles. They become typically nematoid in shape, with the body being flexed twice within the sac. After the fourth molt the males emerge as slender worms. Males are found free in the soil, feeding on roots, and in association with females.

Females, on the other hand, remain attached to the root, cast their second and third cuticles and increase in girth and length until they become pear-shaped or, sometimes, nearly spherical. There is always a protruding neck region. With a suitable host and during warm weather, females begin depositing eggs from 20 to 30 days after entering the host. A female extrudes a "gelatinous" material through the vulva and then deposits the eggs into this material. The gelatinous matrix holds the eggs together in masses and forms a protective covering (Christie, 1959).

Females may be found with the posterior end protruding from the root or just beneath the root epidermis of numerous plants.

No root diffusate or hatching factor is required to stimulate the hatching of Meloidogyne eggs. Hatching requires moisture and oxygen, with the optimal temperature around 28° C.

Males of this genus seem to be functional and sexual reproduction

apparently takes place but has not been reported in the literature. However, males are not always necessary, and parthenogenesis does occur. Tyler (1933) demonstrated that as many as 12 generations could be reared by repeated isolations of single female larvae.

Tylenchulus

Tylenchulus semipenetrans (Cobb, 1914), the citrus nematode, is the only species in this genus whose life history has been studied. It has a very narrow host range, being found mainly on citrus.

The first molt takes place within the egg (Gutierrez, 1947). Van Gundy (1958) noted that the eggs hatch in about 14 days at a temperature of 24° C. He was able to determine the sex of newly hatched larvae by the shorter esophagus and greater body diameter of the male larvae. Of 1,100 eggs experimentally hatched, 26 per cent produced males.

The male completes its fourth molt in approximately 7 days after hatching. It apparently does not feed during this time nor have adults ever been seen in roots. The only increase in size of the adult male is in body length. The stylet is inconspicuous and small, and the esophagus is shriveled and nonfunctional (Van Gundy, 1958).

Van Gundy also noted that female larvae feed externally on roots during all larval stages after hatching. Second stage female larvae do not develop further without feeding. Adult females become sessile on roots with their heads inserted near the pericycle and the body protruding. Eggs are deposited in a gelatinous material and occur in masses. Masses may contain as many as 100 eggs (Baines, 1950). The life cycle

from egg to egg requires about 6 to 8 weeks when the temperature averages 75° F.

Cobb (1914) states that the females of this species are somewhat gregarious as they are found in definite groups on roots.

Rotylenchulus

The life histories and habits of Rotylenchulus reniformis (Linford and Oliveira, 1940), and Tylenchulus semipenetrans (Cobb, 1913) are similar. R. reniformis has a rather wide host range. A molt in the egg was not observed by Linford and Oliveira (1940), but they did observe 3 molts after hatching. Peacock (1956) hatched eggs in distilled water. He noted molting commenced 24 hours to 4 1/2 days after hatching, and was completed in 8 to 18 days. The larvae undergo their development with little increase in size and apparently without feeding. Only the adult females are known to be parasitic. It is not known if the adult males feed. Females become established either completely within roots or with the posterior part of the body protruding. After feeding has commenced, the body widens and becomes kidney shaped. Copulation takes place after the female becomes enlarged (Christie, 1959). Females were observed by Peacock to begin egg laying 10 days after infecting roots. Eggs are laid in a gelatinous matrix in which males are frequently found. The life cycle is completed in approximately 25 days.

Heterodera

In the beginning of his discussion of the life history and habits of

the species of this genus Christie (1959) writes: "Insofar as the matter has been investigated, all cyst nematodes have substantially the same life cycle. The species differ in this respect only in minor details. This life cycle is, in many ways, much like that of the root-knot nematodes." The list of host plants is long and includes many important economic crops.

The first molt takes place in the egg (Hagenmeyer, 1951). The second stage larvae move about freely in the soil until they enter the host plant roots. The area penetrated is usually near the root tips. Most larvae migrate a short distance through the cortex and become established parallel to the longitudinal axis of the root, with the head near the vascular cylinder.

The male undergoes metamorphosis similar to males of the genus Meloidogyne. After increasing in width, the second stage male undergoes the second and third molts. During the third molt the body becomes elongated and typically nematoid. The fourth molt gives rise to the slender adult male.

As with Meloidogyne females there is no female metamorphosis in the genus Heterodera. The female increases in girth after the second molt. After the fourth molt the adult female is lemon-shaped or nearly spherical depending on the species. Usually the entire body of the female is outside of the root with only the head embedded. The cuticle of mature females changes from white to deep brown. The cyst formed by this cuticle protects the eggs from adverse environmental conditions.

Egg deposition varies with the species of this genus. Heterodera schachtii (Schmidt, 1871) deposits from a few to as many as 131 eggs in a

gelatinous matrix (Raski, 1950). Heterodera glycines (Ichinohe, 1952) may deposit up to 200 eggs with as many as 400 still remaining within the female body. The females of H. rostochiensis (Wollenweber, 1923) retain all the eggs within the body (Christie, 1959). When the female dies her body becomes a protective cyst encasing the eggs.

Eggs of some species hatch when moisture, temperature, and oxygen tension are optimal. The conditions vary with the species. However, eggs of certain species hatch only when stimulated by diffusates from the host plant roots. Eggs of some cysts may hatch rapidly or hatching may occur over a 3 or 4 day period.

Raski (1950) found H. schachtii females contained embryonated eggs 31 days after they entered the host plant.

MATERIALS AND METHODS

Sweetgum roots infected with S. whittoni were obtained from the tree parasitized by this nematode on the bank of Hatchet Creek. Roots were placed on a 60 mesh screen and washed with tap water. Mature females and cysts were dissected from the roots under the stereoscopic microscope at 45 x magnification. Cysts washed from the roots were collected from the screen by tilting it and washing the cysts into a beaker with a gentle stream of water. All stages of the nematode were manipulated with a finely drawn out pipette. Some cysts and mature females were stored in tap water in Bureau of Plant Industry Station dishes. Females deposited eggs in tap water and larvae readily hatched from them and from eggs in cysts. There was apparently no harmful effect on the eggs from such additives as chlorine in the tap water. Female nematodes and cysts were fixed overnight in hot (50° C) 2 per cent formalin to be used for further study. Young and mature females were also stained and fixed in hot 0.1 per cent cotton-blue lactophenol after the method of Goodey (1957). Nematodes were placed in the boiling solution for 1 to 3 minutes and mounted in clear lactophenol. This treatment was especially good for studying the development of the reproductive system. There was considerable distortion of many of the organs except the gonads which stained slightly darker than the surrounding tissue and were rendered clearly visible.

Second stage larvae and males were removed from the soil by the

sieving and Baermann funnel technique of Christie and Perry (1951). Larvae were also hatched from eggs held in tap water in BPI dishes and depression slides. These larvae and males being eel-like in form were best manupulated with a finely sharpened bamboo pick. Live larvae and males were studied in tap water in glass depression slides at 430 x magnification under the compound microscope. Larvae and males were preserved in hot formalin and in cotton-blue lactophenol.

Slides were made of living nematodes in water and specimens fixed in 2 per cent formalin and in cotton-blue lactophenol in the following manner. Under the stereoscopic microscope at 45 x magnification nematodes were picked up with a finely sharpened bamboo pick. They were placed in a small drop of water, 2 per cent formalin or clear lactophenol on a glass slide. Three pieces of glass wool approximately the diameter of the nematodes were placed in the drop as supports for the cover slip. An 18 mm. round cover slip was added; the slide was placed on a turntable and ringed with Zut.[1] Prior to sealing the water mounts a drop of the intravitam stains, methylene blue or neutral red, was sometimes placed on the slide on one side of the cover slip and drawn under it by the capillary action of a piece of filter paper held on the opposite side. These stains caused certain structures to stand out more clearly than in the unstained worms.

Live females were studied microscopically in water in depression slides. Because of the varying diameter of the females the cover slip of each slide was supported by 3 small blocks of a mixture of one-half

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[1]Commercial compound used for sealing slides.

vaseline and one-half paraffin wax. The cover slip could be gently pressed down until it touched the specimen and then sealed with Zut.

Paraffin sections of mature females were prepared. Roots with females attached were also prepared and sectioned. The following technique was used.

1. Kill and fix in hot (50° C) 2 1/2 per cent formalin for 24 hours.
2. Add a few drops of thick (20 per cent) celloidin in ether-absolute alcohol solution, at room temperature, every few hours. Leave off the top of the vial in later stages to allow celloidin to thicken. When it can be picked up in a soft lump with forceps, transfer to pure chloroform to harden, cut from forceps, trim to desired size and shape so nematodes will be in center of the lump.
3. Transfer to 1 : 1 solution of glycerin and absolute alcohol to clear. Can be kept here indefinitely.
4. To embed in paraffin for serial sections, wipe off glycerin-alcohol thoroughly and place in 1 : 1 solution of chloroform-paraffin oil in open vial on top of oven or on warming plate (low temperature).
5. Pour melted paraffin into vial, about half full, allow to harden, place lump of celloidin containing nematodes on top of hard paraffin, add enough chloroform-paraffin oil to cover the lump, place in paraffin oven at 56 - 58 degrees, until paraffin melts and celloidin drops to bottom of vial.
6. Change paraffin 3 times to get rid of all chloroform-paraffin oil, then block and section.
7. Affix to slide by floating on solution recommended by Johansen (1940, p. 125): 2 cc. acetone, 1 drop methyl benzoate, 8 cc. HOH, 2 or 3

drops Mayer's or Haupt's adhesive. Place on warm plate to stretch slightly and straighten, drain off excess adhesive, allow to dry, stain with hematoxylin.

8. Mount in permount.

Slides were studied under oil immersion and camera lucida drawings and photographs were made of the specimens. Measurements were made with an ocular micrometer, of various morphological structures. Critical measurements were taken from the camera lucida drawings.

In order to have a continuous supply of specimens in various stages of development, cultures were maintained in the greenhouse. Sweetgum seedlings 2 to 6 inches tall growing in sterile soil in clay pots were innoculated with second stage larvae and cysts. Developing females were removed from the roots as needed for study.

Specific materials and methods are mentioned in the descriptions of the particular experiments where they apply.

Eggs for embryological study were obtained by dissecting mature females and cysts. Eggs were observed in tap water in glass depression slides at 430 x magnification. To determine the length of time required for cleavage to occur, slides were kept in moisture chambers at room temperature and water around the eggs was changed every 24 hours. During observation, eggs were turned by forcing air through a pipette onto the surface of the drop of water in which the eggs were contained. This caused the eggs to turn and thus dorsal, lateral, and ventral views could be obtained.

To study the development of the female a modified technique based on that of Raski (1950) was used. Because preliminary tests indicated

approximately 60 days were required for female S. whittoni to mature, an experiment was planned to extend over a 2 month period. Large numbers of 7-day-old, second stage larvae were mixed into moist, sterile sand. The sand was allowed to stand for 7 more days; then, 120 sweetgum seedlings, germinated in sterile pearlite, were planted in the sand. These were allowed to grow for 48 hours, and all the seedlings were washed free of sand and planted in sterile soil. The plants were maintained at room temperature. On each succeeding day for 2 months, 2 seedlings were washed free of soil and stained with cotton-blue lactophenol according to the method of Goodey (1957). Larvae were removed from the roots and mounted in clear lactophenol on slides for microscopic examination.

In order to study the influence of nematode trapping fungi on the larvae, the following method was employed. Petri plates containing sterile 2 per cent water agar were innoculated with spores from a culture of Arthrobotrys sp. The plates were maintained at room temperature for 10 days. At the end of this time the mycelium of the fungus had covered most of the agar. The species of Arthrobotrys used in these experiments captures nematodes by means of sticky loops which grow out from the hyphae. When the nematodes pass through the loop they become caught.

Second stage S. whittoni larvae were hatched from cysts in tap water. They were rinsed 3 times in distilled water before being used in these experiments.

DESCRIPTION OF THE SPECIES

Sphaeronema whittoni new species

The new species described below has been found attached to sweetgum (Liquadamber styraciflua) roots and in soil around the roots.

Measurements were made of the larvae, mature males, and mature females. Measurements taken were as follows:

L = total length of body in μ

$$a = \frac{\text{total length of body}}{\text{width of body}}$$

$$b = \frac{\text{total length of body}}{\text{distance from anterior end to end of esophagus}}$$

$$c = \frac{\text{total length of body}}{\text{length of tail}}$$

V = position of vulva

$$T = \frac{\text{length of testis}}{\text{total length}} \text{ in per cent}$$

Gub. = length of gubernaculum in μ

Spic. = length of spicules in μ

Female: L = 325 - 650 μ; a = ?; b = ?; c = ?; V = equatorial to subterminal.

Male: L = 390 - 465 μ; a = 32.2 - 39.0; b = ?; c = 7.6 - 9.1;

T = 12.6 - 21.7 per cent; gub. = 5 - 6 μ; spic. = 12.2 - 14.4 μ.

Larva: (Fig. 1A) L = 401.5 - 477 μ; a = 24.0 - 29.8; b = 2.8 - 4.2; c = 8.7 - 13. Body slender, cylindrical, and tapering at both ends. Annules fine, striae less than 1 μ apart. Lateral lines not observed. Excretory pore posterior to the nerve ring. Hemizonid 2 annules anterior to excretory pore. Lip region not offset from head. Cephalic sclerotization weak. Stylet 18 - 19.9 μ long. Stylet tip 47 - 56 per cent of total stylet length. Stylet knobs rounded swellings. Esophagus long and slender. Orifice of dorsal gland 3 μ posterior to stylet knobs. Corpus slender. Median bulb elongate with conspicuous valve. Isthmus well defined. Gland cells overlap intestine. Anus very obscure. Phasmids small and pore-like, 24 - 27 μ from tail tip; very difficult to see; observed on only 3 specimens. Tail tapering to bluntly rounded terminus. Terminus frequently flattened with ventral side longer than the dorsal side.

Female: (Fig. 1C) Body sub-spherical to spherical with protruding neck. Neck usually straight and stout, less than 100 μ in length; may be elongate and variously curved due to influence of the tissues of the host plant surrounding it. Cuticle strong and well developed, up to 10 μ thick. No pattern or markings observed. Lateral lines not observed. Excretory pore, nerve ring, and hemizonid not observed. Lip region with a circum-oral elevation. Cephalic framework weak. Stylet 19 - 21 μ long. Stylet tip 54 - 57 per cent of total stylet length. Stylet knobs rounded swellings. Esophagus well developed. Orifice of the dorsal gland 3 - 4 μ posterior to stylet knobs. Corpus elongate cylindrical. Median bulb large, spherical, and very muscular, and provided with heavily

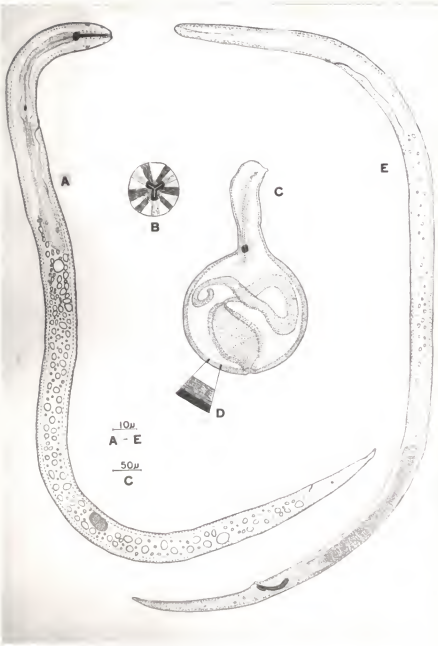

Figure 1. Sphaeronema whittoni. Camera lucida drawings. A: Second stage larva. B: Cross section through median esophageal bulb of female. C: Mature female. D: Cross section through mature female cuticle. E: Mature male.

sclerotized triradiate valve. Isthmus, glands, and junction of esophagus and intestine obscured from view by thick, dense cuticle. Excretory pore not observed. Vulva equatorial to subterminal, and an opening through the thick cuticle, the lips being flush with the body surface. The short vagina leads into a large, conspicuous, thick-walled uterus which becomes distended and packed with eggs.

Male: (Fig. 1D) Labial region smooth, not set off from body. Cephalic framework obscure. Annules very fine. Lateral lines not observed. Hemizonid located 2 annules anterior to excretory pore. Excretory pore 70 - 71 μ from anterior end. Testis 12.6 - 21.7 per cent of length. Spicules 12.2 - 14.4 μ long, slender, slightly curved ventrally. Cuticle protrudes around cloacal opening. Bursa absent. Gubernaculum 5 - 6 μ long, slightly curved. Phasmids not observed. Tail conoid, curved slightly ventrally, with rounded terminus.

Type Host: Roots of sweetgum, Liquidambar styraciflua L.

Type Locality: On bank of Hatchet Creek, 8 miles east of Gainesville, Florida.

Date Collected: October 12, 1958.

Type Specimens: Holotype - female; allotype - male; all types deposited in State Plant Board Collection, Gainesville, Florida.

Differentiation: This new species differs from the type species, Sphaeronema californicum (Raski and Sher, 1952) and S. minutissimum (Goodey, 1958) in the following respects. Female: The females of S. whittoni do not possess protruding vulval lips as do those of S. californicum, but the lips are flush with the body wall. The cuticle of this new species is not marked by a reticulate pattern as is that of S.

californicum. The stylet knobs of S. whittoni are simple, rounded swellings not directed posteriorly as in S. californicum or protruding laterally as in S. minutissimum. The minimal body size recorded for females of S. whittoni is larger than that of the other two species of the genus. Females have been found which were approximately 5 times larger than S. minutissimum and 3 times as large as S. californicum.

Male: S. whittoni males are quite similar to S. californicum males. (Goodey reported no males for S. minutissimum.) However, in S. whittoni, the hemizonid is anterior to the excretory pore while in S. californicum it is located posterior to the pore. The testis of S. whittoni is generally not as long as that of S. californicum. The spicules of this new species are not as long as those of S. californicum, but the gubernaculum is slightly longer.

Larva: Larval body-length measurements overlap those of S. californicum but are greater than those of S. minutissimum. The stylet is longer than that of either of the other two species. The knobs are round swellings similar to those of S. californicum but quite different from knobs of S. minutissimum which are convex anteriorly and flat posteriorly. The labial region is not characterized by a circum-oral elevation as in S. minutissimum larvae. The esophageal glands of S. whittoni overlap the intestine, whereas they do not in S. californicum and Goodey does not indicate overlap in S. minutissimum.

Egg: The egg of S. whittoni is approximately twice as long as wide. The egg of S. minutissimum is almost spherical and much smaller. The size range of eggs of S. whittoni is discussed in the following section on embryology.

EMBRYOLOGY AND THE FIRST STAGE LARVA

Egg

Young, mature females laid a few eggs when they were placed in tap water. These eggs were usually embryonated, being in the 2 or 4 cell stages when extruded. Usually 5 or 6 eggs were passed but most were retained in the uterus of the female. The egg laying habits of S. whittoni are similar to those of Heterodera schachtii as described by Raski (1950).

The eggs of Sphaeronema whittoni are almost twice as long as broad with equally rounded poles. Eggs measure from 75 μ to 80 μ long by 45 μ to 50 μ wide. The ventral surface of the egg is slightly concave and the dorsal surface is convex. The differences in dorsal and ventral sides are rather indistinct on some eggs.

The egg is composed of closely packed globules of different sizes and is bounded by two membranes, the outercovering, or egg-shell, and the inner vitelline membrane. According to Chitwood and Chitwood (1950) a similar arrangement is found in most species of the Tylenchoidea. The egg-shell is a smooth, clear, sticky membrane. When eggs are removed or extruded normally from the female, they will adhere to almost any surface with which they come into contact. Gentian violet stains the shell darkly. After 24 hours in a 10 per cent solution of commercial clorox (.05 per cent Sodium Hypochlorite), egg-shells were found still intact, but the

thickness was greatly reduced. In undiluted commercial clorox (5 per cent Sodium Hypochlorite) egg-shells dissolved completely in 10 minutes. Egg-shells were not visibly affected by 24-hour immersion in concentrated H Cl, 0.2N H Cl, or 0.2N NaOH.

The thin vitelline membrane immediately surrounds the ovum. Before cleavage occurs, it is difficult to see because the globules composing most of the ovum push the membrane against the inner surface of the egg-shell. After cleavage begins the ovum shrinks away from the shell wall and the vitelline membrane becomes visible as it separates from the ovum and the shell wall. The space between the embryo and the vitelline membrane is called the perivitellus space. The extra-vitellus cavity results from the separation of the vitelline membrane from the egg-shell.

The vitelline membrane in nematode eggs has been found to regulate the movement of gentian violet into the egg (Chitwood and Graham, 1940). To test this function in the vitelline membrane of S. whittoni, experiments similar to those of Chitwood and Graham with Strongyloides ratti eggs were carried out. When embryonated eggs of S. whittoni were placed in an aqueous solution of gentian violet the egg-shell stained a dark violet, but the stain did not penetrate the vitelline membrane and stain the embryo. However, after gently heating the egg or exposing it to fat solvents such as acetone or absolute alcohol, the gentian violet penetrated the vitelline membrane and stained the entire egg dark violet.

Two polar bodies can frequently be seen after cleavage has begun in S. whittoni eggs. The polar bodies are located at the anterior end. In some nematode eggs the polar bodies are located near the middle, as in Heterodera schachtii (Strubell, 1888), and in Heterodera avenae

(Wollenweber, 1924 and Mulvey, 1959). In S. whittoni one polar body is located in the perivitellus space and the other nearby in the extra-vitellus cavity.

Embryological Development

No attempt was made to work out cell lineage in my embryological studies. However, the development of the embryo through the 6 cell stage is almost identical with that of Turbatrix aceti (Anguillula aceti) as reported by Pai (1928). The planes of cleavage, sequence of divisions, relative size, and relative position of the blastomeres of the 2 embryos are similar. The development of the embryo of S. whittoni through the 6 cell stage is similar to that of Ascaris megalocephala, as described by Boveri (1899), in most respects except for the shape of the blastomeres, the cells of the embryo of A. megalocephala being somewhat more rounded than those of S. whittoni. Because of the similarities in the early embryos of S. whittoni, T. aceti, and A. megalocephala, I have used the same lettering system to designate blastomeres as Pai, Boveri and others, for the sake of clarity and convenience. Such usage does not imply that the lineage of any cell in the S. whittoni embryo was followed to its ultimate fate.

The protoplasm of the undivided ovum fills the egg membranes (Fig. 2A). This stage is usually designated as (Po) in nematode embryological studies. The first division is transverse to the longitudinal axis of the egg and produces 2 cells of about equal size. The anterior cell is (S_1) and the posterior cell is called (P_1) (Fig. 2B). The nuclei are

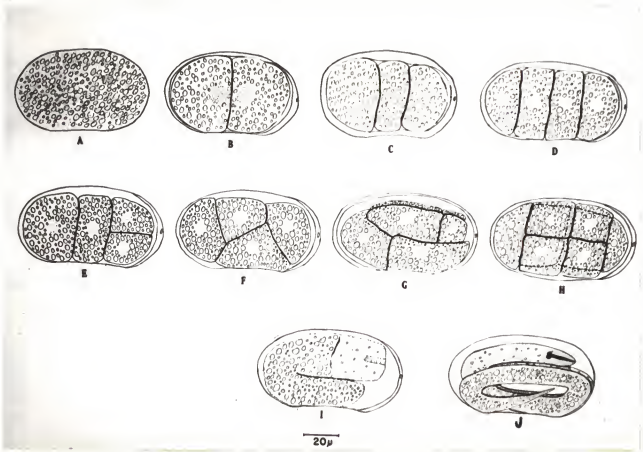

Figure 2. Early cleavages of the embryo. Camera lucida drawings of 1-cell through 6-cell stages A-H. Tadpole stage I. Second stage larva J.

round, clear areas in the protoplasm. The second cleavage division is completed approximately 24 hours after the first, and the plane of cleavage is parallel to that of the first division. The anterior (S_1) cell divides almost equally to give rise to the anterior cell (A) and posterior cell (B) (Fig. 2C) with the resulting cells arranged in tandem. In the third division the plane of cleavage is parallel to that of the first 2 divisions. In the third division, (P_1) divides about equally to form an anterior cell (S_2) and a posterior cell (P_2) (Fig. 2D). This division requires approximately 24 hours for completion. Soon after the completion of this division the 4 blastomeres are arranged in tandem (Fig. 2D). Cell (B) soon shifts to a position dorsal to cell (A) (Fig. 2E). A few hours later the cells have moved into the pattern shown in Fig. 2F. In this situation (A) is anterior, (B) is dorsal, (S_2) is ventral, and (P_2) is posterior. The shifting of the blastomeres is slow and I could not detect any movement. The same was true of the flow of the protoplasm. At the third cleavage the (S_1) cell group, (A) and (B), divides longitudinally to form 4 cells. The 2 on the right are designated by Roman letters, the 2 on the left by Greek letters Alpha and Beta. The embryo is now composed of 6 cells (Figs. 2G and 2H).

From this stage the cleavage rhythm differs in the various cell groups and several cells divide simultaneously. After numerous divisions the ectoderm and entoderm layers are clearly distinguishable (Fig. 3). The light tissue (Fig. 3) at the end of the egg becomes the esophagus. The mesoderm is not visible in side view for it lies as 2 groups of cells lateral to the future gut. No effort was made to trace cell division in the embryo past the 6 cell stage. Due to the small size of the dividing

Figure 3. Embryo with well developed layers of tissue. The light tissue on the left (anterior end) will develop into the esophagus. (Approximately 1000 X)

cells and various cleavage rhythms I could not trace the divisions accurately.

The embryo reaches the tadpole stage after approximately 13 days (Fig. 2I). The first movement was recorded on the twelfth day. The embryo slowly expanded and retracted small areas of the anterior part of the body. The 17-day-old embryo is active and moves around within the egg membranes. By this time it has grown in length until the body is flexed 3 to 4 times within the egg. The stylet tip is apparently the first part of the stylet formed, and it is visible on some 17-day-old embryos. The shaft and knobs are formed during the next 3 days (Fig. 2J).

Hatching

Hatching occurred readily in tap water from the State Plant Board Laboratory, rain water, distilled water, and natural spring water. Hatching was not affected by diffusate from potted sweetgum seedlings. The eggs of Sphaeronema whittoni appear to be highly tolerant to differences in these aqueous media. In contrast, van Weerdt (1959) found the eggs of Radopholus similis would not develop in tap water from the same source as above. He therefore placed R. similis eggs in natural spring water to study their development.

Eggs of S. whittoni hatched within the cysts, or when removed and placed in water in depression slides. When larvae hatched from eggs in a cyst they escaped from the cyst through the vulva or, sometimes, the head region of the female. The latter area, apparently being a weak part

of the cuticle, is more subject to breakdown than the body wall and allows larval exit. However, numerous cysts removed from the soil and cut open were found filled with starved larvae, their intestines being hyaline. The empty egg-shells of these larvae were packed within the cyst. These larvae had apparently not been able to escape from their cysts. Many were dead upon removal from such cysts or they died a short time later. Some whose intestines were opaque, indicating the presence of stored food, survived. Whether S. whittoni larvae can contribute to their own escape from a cyst on which the vulva has become closed or whether they must depend on damage to the wall of the cyst by outside agents in the soil is not known. It is apparent, however, that many hatch and then starve within their cysts because they are unable to escape when the vulva is closed or blocked.

Eggs nearly ready to hatch are distorted in shape as a result of movement of the larvae within. The nematodes become quite active several hours prior to hatching. They move the head from one end of the egg to the other by sliding the whole body along its flexures (Fig. 2J). This vigorous activity causes the membranes of the egg to bulge so that the egg may momentarily lose its characteristic shape. Although the membranes of the egg seem to be subjected to strong pressure from the larval movements, they have never been seen to rupture or break from this pressure alone. The larval activity of the body prior to hatching is accompanied by numerous, strong thrusts of the stylet against the egg membranes. Movement of both body and stylet is interrupted by frequent periods of rest during which the larva is completely immobile. The following observations were recorded on a single egg during a period of

approximately 30 minutes and are characteristic of larval movement. The nematode first moved its head from one end of the egg to the other in a unidirectional manner 3 times. Although the entire body progressed anteriorly during this activity, it was constantly flexed due to the small space within the egg. A period of 5 minutes of inactivity followed. The worm then placed its head perpendicular to the end of the egg and 79 strong stylet thrusts were made in the next minute. The stylet was extruded approximately 3 to 4 μ at each thrust. When the stylet point drove against the egg membranes the latter were momentarily dented in a manner similar to that caused by the pressure of a blunt pencil against one's hand. After the stylet was retracted the membranes regained their normal condition at the site of stylet contact. After a 4-minute rest the larva began movement of the stylet again; this time 43 thrust were counted in the same place. This was followed by 3 one-half minute rest periods interrupted by 3 periods when the stylet was strongly protruded 3 times. The nematode next moved its head to the opposite end of the egg and began intermittent probing action with 1 rest period of 4 minutes and 1 of 7 minutes. It then moved back to its original position and began a 12-minute sustained period of strong thrusts.

This pattern of behavior described in the above paragraph continued for a 5-hour period. The nematode seemed to prefer 1 end of the egg in its stylet activity. It invariably returned to the same spot after switching from 1 end to the other. The egg was left overnight and the larva had hatched by the next morning through a small, round, excentric hole at one end of the egg. As is characteristic of S. whittoni eggs, the shell did not collapse after larval emergence. The above observations

are similar to those made by Dropkin, Martin, and Johnson (1958) on hatching larvae of Meliodogyne arenaria. However, they found that the egg-shells of this species collapse about 1 minute after larval emergence.

I was never able to see any indication that thrusts of the stylet had caused any damage to the membranes of the egg. No secretions from the larval stylet or from elsewhere on the worm were ever observed. Whether the egg membranes are weakened mechanically by the stylet or enzyme action is not known. The thrusts of the stylet were powerful, for when the tip was hindered in its forward motion by the membranes of the egg, the head and anterior part of the body of the nematode were forced posteriorly and the stylet tip was frequently seen to bend.

The opening through which the larva escapes from the egg is quite small. One active larva with its entire body outstretched and moving normally was seen with approximately the posterior one-seventh of its length still encased in the egg-shell. The body was greatly constricted by the boundaries of the aperture through which the anterior portion of the worm had already emerged. The hypodermis and muscle layer were pressed against the intestine. As the diameter of that portion of the body which had passed through the opening was larger than the posterior part, the nematode must have exerted itself greatly to force the body through so small an opening. This specimen was observed for several days in water in a depression slide and it did not free itself of the egg-shell. It is possible that this could have been accomplished had the animal been in soil or some other suitable substrate. No other specimens were seen with the egg-shell squeezing the body as noted in the abnormal case above. The majority of the larvae observed in water were able to

leave their egg-shells without complication.

SECOND STAGE LARVA

Second stage larvae of Sphaeronema whittoni are found in large numbers in soil around the roots of sweetgum trees parasitized by this species. This is the infective stage in the life cycle. After hatching the larvae are quite active and move about freely.

First Molt

The first molt occurs within the egg. It is extremely difficult to see and requires the use of the oil immersion objective to be detected. Even under high magnification, the first molt was seen in only two of the hundreds of Sphaeronema whittoni eggs examined. When the molt was observed, the thin, transparent, cast cuticle could be seen protruding beyond the head of the completely developed larva still within the egg (Fig. 4). There was no indication that the tip of the stylet was shed during the first molt. The molt probably occurs prior to formation of the stylet as Christie and Cobb (1941) found in root-knot nematode larvae. However, van Weerdt (1959) found that the degree of development attained by the first stage larvae of Radopholis similis varied prior to hatching. He found most first stage larvae molted prior to the formation of the stylet, but some molted after the stylet was formed and a molted tip of the stylet was seen attached to the loosened cuticle. It was quite

Figure 4. First stage larva undergoing the first molt within the egg. The old cuticle protrudes beyond the nematode's head. (Approximately 2000 X)

possible that this situation may exist in S. whittoni. Due to the obscurity of the structures, it was not determined if the lining of the amphidial ducts is shed during the first molt in S. whittoni.

Just when and how the larva escapes from the loosened cuticle was not ascertained, but it probably occurs within the egg. Numerous larvae at various stages of development were artificially hatched by applying gentle pressure to the cover slip with a dissecting needle. None of these larvae was enclosed in a loosened cuticle. A thin film of material resembling a molted cuticle was invariably seen within the egg-shells which had held fully developed larvae. The fully developed, artificially hatched second stage larvae were characterized by the presence of globules of unknown composition and origin in the pseudocoelom around the esophagus (Fig. 5). These larvae were otherwise similar to naturally hatched larvae. Some survived artificial hatching and some were observed to undergo further development to become mature males. All larvae liberated from their egg-shells prior to stylet formation died almost as soon as they were free of the egg. These larvae were characterized by their delicate cuticles which were damaged by the slightest pressure. No cast cuticle was ever found in the egg-shell of a well developed, artificially hatched larvae.

Morphology

Certain morphological characteristics have already been discussed in the description of the species.

Figure 5. Artificially hatched second stage larva. Globules in the pseudocoelom bordering the esophagus are characteristic of prematurely hatched larvae. (Approximately 390 X)

External covering

The external cuticle of nematodes serves these animals in much the same manner as does the exoskeleton of arthropods. The cuticle covers the body and lines the mouth, anus, and vulva of nematodes. The shape of the cuticle not only gives form to the body but acts as a flexible protective sheath and place of attachment for internal organs. Internally, the hypodermis is attached to the cuticle and the somatic musculature is attached to the hypodermis. The cuticle is a noncellular layer which, according to Chitwood and Allen (1959), is composed of various scleroproteins. The cuticles of nematodes thus far studied have been found to be layered. For example, Chitwood and Chitwood (1950) state that the cuticle of Ascaris lumbricoides is composed of 9 distinct layers. Hirschmann (1959) found the cuticle in male Heterodera glycines and adults of Hoplolaimus tylenchiformis (Daday, 1905) is composed of 3 layers. The cuticle usually bears transverse striations which may be minute or coarse in accordance with the species. Lateral longitudinal ridges or thickenings marked by grooves may be present and serve as supporting structures. The cuticle may bear various setae and glands.

The exterior of the cuticle of second stage S. whittoni larvae is marked by fine transverse striations or annulations. There were no lateral longitudinal ridges visible. Due to the small size of these larvae the presence of cuticular layering was difficult to determine even under higher magnification. However, the reaction of the cuticle to a 5 per cent solution of sodium hypochlorite indicates it is probably composed of 2 or more layers as shown by the following experiments. When active larvae were placed in a 0.5 per cent solution of sodium

hypochlorite they remained alive for 35 minutes. After 30 minutes the tissues of the esophagus became disorganized and appeared more dense than in normal specimens. The appearance of this tissue was similar to that of formalin-fixed specimens. Thirty-five minutes after treatment there was no larval movement and the animals were dead. One hour after treatment the intestinal globules of the nematodes had coalesced. In some specimens the base of the stylet tip was evenly and smoothly detached from the anterior end of the stylet shaft. The cuticle of specimens treated with 0.5 per cent sodium hypochlorite was not visibly affected.

Larvae placed in a 5 per cent sodium hypochlorite solution were drastically affected within a few minutes. First, a few larvae extruded several small globules of clear material through the anus. The effect of the reagent on the cuticle was quite striking. What was apparently the outer layer rapidly splintered into thin strands sometimes the length of the animal's body. These strands remained attached posteriorly but pulled away from the anterior part of the body. The cuticular layer adjacent to the damaged layer remained intact. A nematode damaged in this manner reminds one of a partially peeled banana. After the sodium hypochlorite entered the animal's body the internal structures broke down leaving the thin cuticular sheath.

When S. whittoni larvae were heated for 10 minutes at 65° C prior to treatment with 5 per cent sodium hypochlorite the splintering phenomenon occurred as it had with unheated larvae. However, in heated specimens the inner cuticular layer remaining after splintering of the outer layer had occurred was also destroyed. Chitwood (1938) conducted experiments on the cuticle of Ditylenchus dipsaci in which he found the outer layer

of the cuticle to be a thermolabile membrane which governs permeability. He found this outer layer was not destroyed by sodium hypochlorite unless the specimens were heated to 65° C for 10 minutes prior to treatment. Hirschmann (1959) treated males of Heterodera glycines and adults of Hoplolaimus tylenchiformis with 5 per cent sodium hypochlorite. She, too, found the outermost layer was not destroyed in the two species she tested unless the specimens had been heated prior to treatment.

S. whittoni larvae were killed after approximately 30 minutes exposure to 0.2N HCl. Ten minutes after immersion in the acid the worms began to coil tightly. Most movement was restricted to the posterior third of the body. Vacuoles formed in the pseudocoelom near the stylet and the intestinal globules coalesced. The cuticle became separated from the hypodermis in the anterior portion of the body after approximately 40 minutes in the acid. The body next became shrunken and distorted. The cuticle was not broken down or dissolved by 0.2N HCl.

Larvae immersed in concentrated HCl were killed almost immediately and the intestinal globules coalesced. After 2 hours the cuticle was detached from the body in most areas. The cuticle extended outward from the body in large bulges. This probably resulted from the cuticle being separated from the hypodermis and/or the somatic muscle layer.

Cephalic region

The labial region is not set off from the body. There are 6 small lips which are difficult to see in face view. The amphidial openings are shall pores at the base of the lateral lips. I was not able to see any labial papillae but these structures may be present though small and

difficult to see. The contour of the lip region is not smooth and rounded in lateral view but is characterized by a shallow V-shaped indentation at the stoma.

The cephalic framework is so weak and obscure on second stage S. whittoni larvae that little of its structure could be determined.

Cephalids. In morphological studies on males of Heterodera glycines and H. trifolii Hirschmann (1956) noted a previously unrecorded structure in the head region. She briefly described it as a biconvex band extending around the body between the cuticle and the hypodermal layer. She studied this structure thoroughly (Hirschmann, 1959) and found that there are 2 such bands a few body annules apart in the head region of Hoplolaimus tylenchiformis and several species of Heterodera. In her earlier studies she had observed only the posterior, or second, band from the anterior end of the body. In 1959, Dr. Hirschmann suggested the term "cephalid" for each of these rings. The one closest to the lip region is the anterior cephalid and the posterior cephalid is located a short distance to the rear of the first. In her 1959 paper, Hirschmann states that the hypodermal chords begin at the anterior cephalid and the 2 median chords start at the posterior cephalid She comments that the biological function of the cephalids is not understood. They may have some sensory function or arise from attachment of organs to the body wall.

Two cephalids are present on second stage larvae of S. whittoni. These structures are visible with an oil immersion lens on most living larvae and can almost always be seen on specimens stained with cotton-blue lactophenol. In these larvae the lateral hypodermal chords have their beginning where the anterior cephalid circles the body and the dorsal and

ventral chords begin where the posterior cephalid circles the body. These relationships can be seen on specimens in a direct dorsal or ventral view and a direct lateral view. This is consistent with Hirschmann's finding in the species in which she studied the cephalids. In S. whittoni the anterior cephalid is located 5 μ from the anterior end of the body and the posterior cephalid is 13 μ from the anterior end.

Hemizonid

The small hemizonid of second stage S. whittoni larvae is located 2 body annules anterior to the excretory pore. When specimens are viewed in a lateral position it is a structure, biconvex in section, located between the cuticle and the hypodermal layer. Goodey (1951) states that the hemizonid of nematodes he studied could be seen extending around the ventral side of the body and ending approximately at the lateral field on either side. Hirschmann (1959) found the hemizonid on nematodes she studied had approximately the same diameter. Because of the small size of S. whittoni larvae I would see the hemizonid only on specimens in lateral view. The structure was only visible in optical section and I could not determine whether it extended up to the lateral field on either side of the animal.

Digestive system

Stylet. The stylet of the larvae is from 18 - 19.9 μ long. The tip of the stylet is 47 - 56 per cent of the total length and has stylet knobs which are characteristically rounded swellings. In overall appearance the stylet is rather strong and stout (Fig. 10). It seems likely from the effects of certain reagents on the stylet that the stylet tip is

composed of different material from the shaft and knobs. Because of dissolving action of NaOCl, the stylet tip and shaft became evenly and cleanly detached as though they had been cut apart with a sharp instrument when larvae were treated in 0.5 per cent NaOCL for 2 hours. The stylet shaft and knobs rapidly dissolved when the worms were treated with 5 per cent sodium hypochlorite. The tip persisted for some time. The stylet was not visibly affected by 0.2N HCl. Fifteen minutes treatment of the larvae with concentrated HCl caused the shaft and knobs to change from opaque to quite translucent in appearance. The shaft shrank slightly in length and had sharp transverse ridges encircling it. After 5 hours the shaft and knobs were completely dissolved with the tip showing no effects from the acid.

Exposure of larvae to boiling distilled water for 1 minute did not visibly affect the stylet. When larvae were placed for 1 minute in hot 0.1 per cent cotton-blue lactophenol or clear lactophenol the shaft and knobs of the stylet were affected in a manner similar to those of nematodes treated with concentrated HCl. The shaft and knobs became translucent and distorted. The shaft frequently resembled the letter Z in shape. The shaft and knobs of the stylet of larvae mounted in clear lactophenol were dissolved and no longer visible approximately 2 months after the slides were made.

Esophagus. The esophagus or, properly, the pharynx, is typically tylenchoid. It measures from 100 μ to 143 μ in length. The slender procorpus is attached to the stylet knobs and appears to be muscular. It is approximately 40 μ long and approximately 5 μ wide. The median bulb is rather slender, being from 7 - 10 μ wide and approximately 14 μ long.

The musculature of the median bulb is well developed and the sclerotized valve is prominent. Posterior to the median bulb is a well defined isthmus which merges into the 3 esophageal gland cells. The boundaries of these 3 cells are obscure but the nuclei are large and conspicuous. The gland cells overlap the anterior portion of the intestine. The lumen of the esophagus is well defined but its junction with the intestine is obscure and was not observed. The orifice of the dorsal gland was obscure in most specimens examined even under 970 x magnification. On specimens in which it could be seen, the orifice was 3 μ posterior to the stylet knobs. The orifices of the two subventral esophageal gland ducts leading into the esophageal lumen in the corpus is a characteristic typical of the tylenchoid esophagus. The openings of these two ducts were never observed in S. whittoni and if present must be of small size.

Intestine. The straight tubular intestine of the larvae is not characterized by any unique features. As in most soil-inhabiting nematodes the intestine of S. whittoni larvae is attached anteriorly to the base of the esophagus. Throughout most of its length it lies free in the pseudocoelom. Posteriorly, the intestine is connected to a small rectum which opens externally through the anus. The anus is an obscure transverse slit in the cuticle. The intestine is packed with dark globules in newly hatched and feeding larvae. As is also the case with most soil nematodes, these intestinal globules are depleted in starved specimens and the intestine becomes translucent. When S. whittoni larvae were treated with heat, acid, alkali, or placed in lethal concentrations of sodium chloride, the intestinal globules coalesced and the worms died. Nematodes treated with NaCl sometimes could be revived by being placed

in tap water, if the coalescence was not very extensive. This is consistent with the observations made by van Weerdt (1959) on the reactions of Radopholus similis to various concentrations of sodium chloride.

Excretory system

According to Chitwood and Chitwood (1950) the tylenchoid excretory system is asymmetric, being composed of a well developed terminal duct leading posteriorly to a canal in one of the lateral (usually the left) chords. The canal may occasionally be in the right lateral chord. This position varies not only with species and strains but also among members of a population. The canal extends both anteriorly and posteriorly from the junction with the terminal duct. The terminal duct has a small nucleus in its wall and the lateral canal has a large sinus nucleus.

In S. whittoni second stage larvae the conspicuous terminal duct empties externally through a small round excretory pore. This pore is located on the ventral side of the animal just posterior to the hemizonid. After extending dorsally from the pore for approximately 3 μ the terminal duct can be seen extending posteriorly until its course is obscured by the anterior end of the intestine. In live specimens treated with neutral red and methylene blue to accentuate the structures of the excretory system, no structure other than the terminal duct could be seen. This was probably because of the small size of the larvae. If the terminal duct does connect with a canal in one of the lateral chords, as is characteristic of the tylenchs, the connection could not be seen in S. whittoni larvae.

Nervous system and sensory organs

The nerve ring encircles the isthmus of the esophagus just posterior to the corpus. It is approximately 7 μ wide. It can be seen on living specimens under 970 x magnification as a transparent band around the esophagus. Because of the small size of the larvae no attempt was made to study the nerves connected to the nerve ring.

The amphidial openings are small round pores at the base of the lateral lips. No amphidial pouches could be seen.

Phasmids are very obscure on these larvae and were seen only in ventral view on only 3 of the hundreds of specimens examined. Each phasmid appeared as a short duct opening laterally through a tiny round pore approximately 25 μ from the tail tip. The right and left pores were not directly opposite one another. One usually was approximately 3 μ anterior or posterior to the other.

No labial papillae were observed on the specimens studied.

Reproductive system

The genital primordium in newly-hatched second stage larvae consists, as it does in first stage larvae, of four cells. The sexes cannot be distinguished at this stage. This group of 4 cells measures 11 μ by 6 μ in size. It is located on the ventral side of the animal 120 μ from the tail tip. The genital primordium is best observed in specimens seen in lateral view. There is no increase in the size of the genital primordium until molting begins.

Sex ratio

No visible morphological differences between the sexes of second

stage S. whittoni larvae could be detected. When the larvae were kept in tap water the sexes could be separated only when the male larvae began the changes associated with the second molt. Because female second stage larvae underwent no development in the absence of food, molting in tap water was indicative that a larva was male. Of 1,583 second stage larvae, 1,235, or 78 per cent, remained unchanged and were presumably female. Further observations confirmed these counts and established the sex ratio to be 22 per cent male and 78 per cent female.

Physiology

Response to dessication

Some of the Tylenchida are able to resist dessication for long periods of time. Christie (1959) revived Aphelenchoides ritzemabosi (Schwartz, 1911) in chrysanthemum leaves dried for 16 months by moistening the leaves. Fielding (1951) found viable larvae of Anguina tritici (Steinbuch, 1799) in wheat galls which had been in dry storage for 28 years. When larvae of Ditylenchus dipsaci (Kühn, 1857) are subjected to unfavorable conditions of moisture, temperature, or toxic materials, they congregate in masses called "curds" or "nema wool." These gray-white masses are large enough to be seen with the naked eye. These massed larvae pass into a quiescent state in which they are resistant to dessication and other adverse environmental conditions. When the curds are within plant tissue the larvae can withstand long periods of drought. Fielding also revived D. dipsaci larvae from the heads of fuller's teasel held in storage for 23 years. Steiner and Albin (1946) recorded

what is probably the longest period of survival of a nematode in dessicated plant material. From a 39-year-old herbarium specimen consisting of a rye seedling leaf they revived 5 specimens (2 females and 3 larva) of Tylenchus polyhypnus (Steiner and Albin, 1946).

Experiments were conducted to study the response of second stage S. whittoni larvae to dessication. In the first experiment, 25 second stage larvae of various ages were placed singly in a drop of water on a depression slide and all but a thin film of water was removed with a fine pipette. In order to insure rapid evaporation, the slides were not covered. The reactions of the worms were observed under 430 x magnification. As long as a thin film of water remained over the animals they moved normally. As soon as the rapidly evaporating film of moisture was gone the larvae underwent a series of abrupt and drastic changes. All movement ceased and the body became shrunken and distorted. If water was added to the slide before the worm was too badly damaged the animal sometimes became active again. In most cases the changes were irreversible. The dead nematodes were shrunken, distorted, and the intestinal globules had coalesced.

In the second experiment, 25 second stage larvae of various ages were placed singly in a drop of water on a depression slide. Each slide was placed on glass supports in a covered petri dish containing a small amount of water. The dishes were kept at room temperature. At the end of approximately 2 weeks the moisture on the slides and in the dishes had evaporated and the nematodes were dead. None revived when moistened.

None of the isolated S. whittoni larvae survived the rapid or slow drying under the conditions of these experiments.

To test the effects of drying on grouped larvae, 4 groups of 25 larvae were placed on depression slides and most of the water was removed as in the first experiment. None of the larvae could be revived in 3 of the slides. Three individuals on the fourth slide were revived and lived a short while. These 3 were beneath the other larvae in their group and were thus apparently kept somewhat moist while those on the outside of the group dried.

In the final experiment, 4 groups of 25 larvae were treated as the single larvae were treated in the second experiment. After approximately 2 weeks when the slides were dry no worms in any of the 4 groups could be revived.

These experimental results indicate that the second stage larvae of S. whittoni lack the ability to withstand dessication under the conditions of these experiments. This was confirmed by trying to revive specimens which had been dried in soil. They also failed to revive.

Response to temperature

The use of heat to kill nematodes infecting plants is one of the oldest control methods used against these plant parasites. The thermal death points of many plant parasites have been studied. Christie (1959) discussed the amount of heat and time of exposure necessary to kill various plant parasites within plant tissues without damaging the host. Lethal temperatures to various nematode species ranged from 43° C for 30 minutes to 54.4° C for 60 seconds. The effect of temperature on the life cycle of Radopholus similis was studied by van Weerdt (1959). He reported 24° C to be the optimum temperature for development of this species.

The response of second stage S. whittoni larvae to 4 different temperatures was studied. Facilities were not available for studying the response of the nematodes to a wide range of constant temperatures only a few degrees apart. Therefore, the 4 available temperatures used were widely separated. Four groups of 100 12-hour-old larvae were held at 5° C, 24° C, 32° C, and 45° C, respectively.

Ninety-three nematodes survived for 45 days at 5° C. The nematodes were immobile while in water at 5° C, but slowly became active when warmed to room temperature (24° C). Body movements during warming were quite characteristic. The first movement usually noted was the bending of the posterior quarter of the body ventrally to approximately 90° to the main body axis followed by a slow straightening. This was usually repeated several times. The worms then slowly formed the approximate middle half of the body into an elongate S with the anterior and posterior quarters of the body held along the line of the longitudinal axis. After approximately 30 minutes of this type movement the animals moved in a normal undulating motion.

None of the larvae held at 5° molted. When the larvae died, the intestines still contained numerous globules. This would indicate death probably did not result from depletion of stored food in the gut.

Ninety-five of the larvae in the group held at 24° C lived more than 60 days. Five died at the end of the first 30 days of the experiment. Twenty of the 95 larvae which survived 60 days underwent 3 molts to become mature males. Larvae were active during most of the course of the experiment. The nematodes which survived the 60-day period had very few globules within their intestines. The remaining 70 died, apparently as

a result of starvation because the intestines of dead worms were hyaline.

None of the 100 larvae held at 32° C survived more than 30 days. No molting occurred in any of the larvae. Prior to death, larvae had globules within their intestines but the tissues in the anterior end of the body were abnormal in appearance, being more opaque than in healthy specimens. There were small vacuoles within the body cavity in this region. Death of the nematodes held at 32° C was apparently due to damage caused by the temperature and not a result of starvation as the intestines contained many globules.

All larvae held at 45° C died after approximately 10 minutes exposure to the heat. These specimens were damaged in the same manner as those held at 32° C. The exposure to 45° C for 10 minutes also caused extensive coalescence of intestinal globules.

The results of these experiments are summarized in Table 1.

TABLE 1

SURVIVAL TIME OF LARVAE
HELD AT FOUR TEMPERATURE LEVELS

Temperature	Time of Survival	Molting of Males
5° C	45 days	-
24° C	60 days	+
32° C	30 days	-
45° C	10 minutes	-

As the data in Table 1 indicate, 24° C was the most ideal temperature of those tested for the larvae. They survived longest at this temperature and the male larvae molted. Death of larvae at 24° C was apparently due to starvation as indicated by the depletion of intestinal globules. Death of larvae at 5° C, 32° C, and 45° C was possibly a result of adverse temperature as starvation was not indicated by the absence of globules in the intestines of the dead worms. Molting apparently could not occur at temperatures as low as 5° C and as high as 32° C although the larvae lived long enough to molt. Because of the rapid killing at 45° C, molting could not have occurred at this temperature. Had further temperature studies been conducted, the range of temperatures around 24° C, at which larvae are not damaged by temperature effects, could probably have been ascertained.

Experiments were conducted to determine if larvae could withstand freezing or if they would be, like many organisms, fatally damaged by it. Larvae were subjected to freezing in tap water in BPI dishes in the refrigerator for periods of 15, 30, and 60 minutes. A few larvae could be revived for a short while after having been frozen 15 minutes. However, they lived only a few minutes. None could be revived after 30 and 60 minutes freezing.

Response to osmotic concentration

Because soil nematodes inhabit the moisture phase of the soil, and this soil moisture contains various dissolved salts and other substances, nematodes come into contact with varying osmotic pressures. Machmer (1958) conducted field experiments in citrus and papaya plantings in which

he studied the effect of tolerable soil salinity on nematode population numbers. He reported nematodes in salt-treated plots to be 3 or more times as numerous as in the check plots. Twenty times more Tylenchulus semipenetrans were recovered in the upper 2 1/2 inches of soil in the salt-treated plots than in the checks. The tolerance of Radopholus similis to various osmotic concentrations of NaCl in vitro was investigated by van Weerdt (1959). He found this species could not tolerate NaCl concentrations higher than 2 per cent. At 2.5 and 3 per cent the animals were visibly affected and some were killed after 48 hours. Most specimens could be revived when transferred to deionized water. The nematodes were always killed at concentrations of 4 per cent NaCl and above.

The following experiment was conducted to study the reactions of second stage S. whittoni to various concentrations of NaCl and to learn the range of NaCl concentrations in water tolerated by the larvae. Ten different concentrations of NaCl from 0 to 1.0N as shown in Table 2 were prepared using deionized water. One ml of each of these concentrations was added to a BPI watchglass. The watchglasses were then placed on moist filter paper in petri dishes and 10 active larvae were put in each solution. Four replicates were made of each concentration. Observations were made of the larvae under the stereoscopic microscope after 1, 6, 18, 24, and 48 hours. Individuals showed some difference in reaction to the same osmotic concentration of NaCl but there was no apparent difference between replicates.

Because S. whittoni did not survive the lowest (0.1N) concentration of NaCl beyond 6 hours, a similar experiment was conducted to determine

LEGEND FOR TABLE 2

(+) Indicates no visible effect of NaCl. Larvae moving normally when compared to controls.

(o) Indicates that movement was absent or slight. Vacuoles present in body cavity near anterior end of esophagus. Bodies sometimes curled, often somewhat shrunken. Some coalescence of intestinal globules. Larvae in this category were revived when placed in deionized water.

(-) Indicates no movement. Larvae relaxed with bodies outstretched and shrunken. Vacuoles present in anterior portion of body cavity. Intestinal globules coalesced. Larvae failed to revive when transferred to deionized water.

TABLE 2

REACTION OF S. WHITTONI LARVAE
TO INCREASING CONCENTRATIONS OF AQUEOUS NACL SOLUTIONS
CORRELATED WITH TIME

Concentrations of Aqueous NaCl by Normality	Time in Hours				
	1	6	18	24	48
0.0	+	+	+	+	+
0.012	+	+	+	+	+
0.025	+	+	+	+	+
0.05	+	+	+	+	+
0.1	+	o	-	-	-
0.2	+	o	-	-	-
0.3	o	o	-	-	-
0.4	o	o	-	-	-
0.5	o	-	-	-	-
0.6	o	-	-	-	-
0.7	o	-	-	-	-
0.8	o	-	-	-	-
0.9	-	-	-	-	-
1.0	-	-	-	-	-

tolerance to lower concentrations. In the second experiment the concentrations of NaCl used were: 0.05N, 0.025N, and 0.012N. The results of these two experiments are combined in Table 2.

The data in Table 2 indicate that S. whittoni larvae have little tolerance for NaCl concentrations higher than 0.05N NaCl. Nematodes were strongly affected by 0.1N NaCl and were killed after 18 hours.

Response to drugs

Considerable work has been done, particularly in England, on the effect of root exudates, and certain chemicals isolated from them, on the hatching of various cyst nematode eggs. Work has also been done on the action of materials as attractants to larvae. However, little has been done in the way of studying the effects of various drugs on the larvae themselves. I found no reports in the literature of experiments in which soil-inhabiting nematodes were treated with various drugs and their reactions studied. Therefore, I thought it would be interesting to treat second stage S. whittoni with certain drugs known to affect animal nervous systems.

Larvae were treated at room temperature with aqueous solutions of adrenalin, acetylcholine, atropine, nicotine, and strychnine sulfate. The following procedure was used to test the larvae in each of these materials. Ten second stage larvae of various ages were placed with a pick in a drop of the test material in a depression slide and their reactions observed through the compound microscope using 430 x magnification. Ten larvae were placed in a drop of tap water and served as controls. Each experiment was repeated 3 times.

Adrenalin. Larvae were treated with an adrenalin solution of 1 part per 1000. The nematodes began reacting to the adrenalin within 1 minute after the experiment began. The following behavior of 1 larva was typical. The animal began to coil its body, at first rather loosely but with the coils becoming tighter. Then the body was straightened and a series of jerky movements occurred similar to those exhibited by worms just prior to death from gentle heat. This behavior continued for approximately 12 minutes. After this, the worm straightened the body. The extreme anterior portion was then turned sharply so that the head was directed posteriorly. The head was further turned until it was held against the body approximately at right angles. (This configuration in which the body was straight and the extreme anterior part formed a tight loop was peculiar to adrenalin-treated larvae.) The animal remained in this position with only an occasional slight jerky movement of the body for approximately 3 minutes. After this the body became straightened and the anterior end continued to jerk slightly from side to side. After 20 minutes in the adrenalin the nematodes ceased to move. All had vacuoles in the body cavity at the anterior part of the body. These vacuoles first became visible as small round bubbles in the body cavity at the level of the knobs of the stylet. They slowly increased in size becoming approximately 5 μ wide and 10 μ long. Three such vacuoles usually formed in tandem and moved slowly in a posterior direction. When nematodes were transferred to tap water for approximately 20 minutes they began moving normally and the vacuoles slowly decreased in size until no longer visible. The cause of the formation of these vacuoles is not known. Similar vacuoles were noted in larvae treated with certain materials other

than adrenalin.

The visible results of the influence of adrenalin on larvae in this experiment were abnormal body movements and the appearance of vacuoles in the body cavity. The abnormal movement noted in the body apparently resulted from the adrenalin's influence on the somatic muscles or nerves innervating the somatic musculature.

Untreated larvae were stained to detect the presence of adrenalin in the tissues using the method suggested by Gomori (1952). Fresh larvae were fixed in Regaud's mixture (10 per cent formalin containing 5 per cent $K_2Cr_2O_7$) and mounted on slides for examination. Light brown shaded areas indicated the presence of adrenalin. Such coloring in the somatic muscle sheath was more prevalent in the anterior region of the body but was present throughout the entire length. There was no positive reaction for adrenalin in the nerve ring of stained larvae.

Acetylcholine. Ten minutes after being placed in an acetylcholine solution of 1 part per 1,000,000 the larvae became quiescent with their bodies loosely coiled or S-shaped. After approximately 25 minutes in the solution each larva began slow stylet thrusts. The tip of the stylet was protruded about 3 μ at each thrust. Small vacuoles similar to those noted in adrenalin-treated larvae formed. Forty-five minutes after the experiment began the stylet thrusts averaged one every 10 seconds or 6 per minute. Fifty-five minutes after the experiment began the stylet thrusts were occurring at the rate of 30 per minute. Periods of stylet activity averaged 3 minutes in duration followed by rest periods of about 1 minute. Some jerky movement and body coiling were noted. Larvae transferred to tap water for approximately 1 hour lost the vacuoles in the

body cavity, ceased movement of the stylet, and moved normally.

The most obvious influence acetylcholine had on the larvae was to cause the stylet protractor muscles to contract. This resulted in movement of the stylet. The visible influence on somatic musculature resulted in the nematodes becoming quiescent rather than abnormally active. There was no visible effect on the muscles of the esophagus. However, due to the obscure appearance of the esophageal structures in S. whittoni larvae, contraction of esophageal muscles could have been overlooked. Therefore, it seemed desirable to repeat the experiment using a larger plant parasitic nematode to determine the effects of acetylcholine on esophageal muscle. Adult female Radopholus similis were chosen and treated with acetylcholine. In this species the stylet movements began after only 7 minutes in the drug. At first the thrusts occurred at the rate of 53 per minute. After 25 minutes the rate had increased to 64 per minute and after 45 minutes the thrusts were occurring at the rate of 70 per minute. The R. similis specimens formed themselves into loose coils as the S. whittoni larvae did. There was no visible activity of the esophageal musculature of R. similis. As with S. whittoni, the most obvious visible effect of acetylcholine on R. similis was the projection of the stylet.

Atropine. S. whittoni second stage larvae were apparently not much affected by treatment with 0.5 per cent atropine. Body movement seemed to have been slightly slowed but no evidence was seen of any other effects. Results of experiments in which acetylcholine-treated larvae were transferred to atropine were inconclusive.

Nicotine. S. whittoni larvae were rapidly and profoundly affected by

0.2 per cent nicotine sulfate. The following observations made on 1 worm were typical. Within approximately 1 minute after contacting the nicotine sulfate the larva began thrusting the stylet outward. During the second minute the stylet made 17 thrusts and failed to retract to its normal position after the last thrust. The tip was extended about 3 μ beyond the head. The protractor muscles contracted 5 more times causing the already projected stylet to move outward very slightly. The stylet retracted slightly after each of these muscular contractions. After 7 minutes of inactivity the stylet was gradually retracted to its normal position. The stylet immediately began a period of rapid and strong thrusts, each returning to its normal retracted position, which lasted for approximately 1 minute and 30 seconds. The rate was about 150 thrusts per minute. After the last thrust the stylet tip remained protruded and the protractor muscles continued to contract for 10 minutes. The larva was transferred to tap water and after about 3 minutes the stylet returned to its normal position in the body. No further stylet movement occurred. Some larvae were observed to thrust the stylet rapidly as many as 400 times before it failed to retract. When this stage was reached the larvae were always able to retract the stylet to its normal position if they were removed from the nicotine sulfate and placed in tap water.

Larvae in nicotine sulfate moved in their usual undulating manner but somewhat faster than normal.

The musculature of the esophagus appeared to be affected by nicotine sulfate. As nearly as I could determine the muscles seemed to move in an uncoordinated manner. In order to further investigate the effect of this

drug on esophagi of nematodes, two larger species of nematodes were treated with nicotine sulfate. Adult females of Radopholus similis and Rhabditis sp., a saprophyte, were used because of their large, well-developed muscular esophagi.

R. similis treated with nicotine sulfate exhibited the same type of stylet activity as was noted in treated S. whittoni larvae. The principle region of the esophagus affected was the muscular median bulb. Muscle fibers in various parts of this bulb contracted in such a manner as to cause continuous uncoordinated movement of the muscles in the bulb. Thus, the valve did not open and close rhythmically as it does in normal feeding activity, but jerked in various directions by the uncoordinated contractions of the muscles attached to it.

When Rhabditis sp. females were placed in nicotine sulfate the esophageal muscles became quite active. The normal peristalsis of the anterior part of the esophagus was replaced by uncoordinated twitching of the muscle fibers. The basal bulb and valve were affected in much the same way as the median bulb of R. similis.

The most significant effect of nicotine sulfate on S. whittoni larvae, and other stylet-bearing nematodes treated with it, was the stimulation to secrete a material, possibly saliva, through the stylet. The occurrence of this reaction possibly depended on the condition of the animal at the time of treatment because it was never observed to occur in all the treated nematodes in 1 replicate. In 1 replicate of 1 experiment, 9 of 10 larvae treated with atropine then nicotine sulfate secreted material from the stylet freely. This experiment was repeated but the phenomenon was never observed on more than 40 per cent of the

treated animals.

After leaving the stylet the secretion took the form of a thin hyaline thread with rounded enlarged areas on it, like a string of beads. Secretion began approximately 5 minutes after exposure to nicotine sulfate and continued for approximately 3 minutes. The material could be seen slowly issuing forth from the stylet tip. The length of the strand varied with individuals, but it sometimes became 15 μ long. It usually became coiled and tangled with increasing length. The material retained its form in the nicotine sulfate and in tap water. It remained attached to the stylet for 3 or 4 days.

Strychnine. S. whittoni larvae reacted to 0.5 per cent strychnine sulfate after approximately 30 minutes in the solution. The body movements increased in rapidity and were characterized by abnormal jerking. The anterior portion of the body was particularly active. There was no visible effect on either stylet or esophageal musculature. After 1 hour in this material the larvae became quiescent. They revived after transfer to tap water.

Attachment to Roots of the Host

Dropkin (1957) studied the relationship between period of contact of the host plant and the entrance of Heterodera rostochiensis larvae. He used larvae less than 24 hours old and found that about 80 per cent of the larvae entered by the end of 48 hours after contact and that little or no entry occurred after that time. Exposure of seedlings to single larvae yielded results comparable to exposures to groups of 25

larvae.

Experiments were conducted to determine the minimum age at which S. whittoni second stage larvae will enter sweetgum roots. Sweetgum seeds were sterilized by placing them for 1 minute in a 1 per cent solution of 8-hydroxy-quinoline-sulfate solution. Seeds were germinated in moist pearlite in a moist chamber. Germination required approximately 14 days at room temperature. Larvae hatched within the previous 24 hours from cysts held in BPI dishes containing tap water were used in these experiments. Seedlings with lateral roots 1 to 2 cm. long were transferred singly to petri dishes containing moist filter paper. Larvae were collected with a micropipette and transferred to a drop of water on a cover glass. The cover glass was placed in the petri dish so that the roots entered the drop of water. Fine sand was sprinkled to cover the drop of water on the root. The sand was slightly moistened and the cover replaced on the petri dish, which was left undisturbed for varying periods of time. Roots were gently washed free of sand and examined under 112 x magnification. Roots were next stained in 0.1 per cent cotton-blue lactophenol, cleared in lactophenol and examined again.

In the first experiment 5 seedlings, each inoculated with 1 larva, were examined after 24, 48, 72, 96, 120, 144, and 186 hours. No larvae were found attached to the roots. The experiment was repeated using 20 larvae per seedling. The results of the second experiment were also negative. A third experiment was conducted using 20 larvae per seedling and each time of exposure was represented by 3 seedlings. Seedlings were examined at daily intervals from 8 to 20 days. The results of this experiment are recorded in Table 3.

TABLE 3

ENTRANCE OF LARVAE IN CONTACT WITH SWEETGUM ROOTS FOR VARIOUS PERIODS. LARVAE WERE ONE DAY OLD WHEN FIRST PLACED IN CONTACT WITH ROOTS.

Days of Contact Between Plant and 20 Nematodes	Number of Larvae Attached to Roots			
	Seedling No.			Row
	1	2	3	Total
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	1	8	5	14
16	3	1	5	9
17	0	6	2	8
18	4	4	5	13
19	6	2	4	12
20	5	3	1	9

The data in Table 3 indicate that larvae in this experiment did not attach to host roots until 15 days after inoculation. After the fifteenth day larvae were found on all seedlings removed and examined up

through the twentieth day.

In the next experiment larvae were held in tap water in BPI dishes for 14 days before being used to inoculate seedlings. Each of 9 plants were inoculated with 20 14-day-old larvae. Three plants were removed on the first, second, and third days after inoculation. The results of this experiment are in Table 4.

TABLE 4

ENTRANCE OF LARVAE IN CONTACT WITH SWEETGUM ROOTS FOR VARIOUS PERIODS. LARVAE WERE FOURTEEN DAYS OLD WHEN FIRST PLACED IN CONTACT WITH ROOTS.

Days of Contact Between Plant and 20 Nematodes	Number of Larvae Attached to Roots			
	Seedling No.			Row Total
	1	2	3	
1	0	0	0	0
2	3	5	2	10
3	1	5	3	9

The data in Table 4 reveal that no larval penetration occurred the first day after inoculation. Penetration did occur on the second day. From this and the preceding experiments, it seems probable that S. whittoni second stage larvae must be approximately 2 weeks old before penetration of roots of the host occurs. The reason for this is not known. Examination of numerous larvae between the ages of 1 day and 14 days revealed no morphological differences. Perhaps the larvae must

undergo some maturation process before they become infective.

Under the conditions of these experiments, the greatest number of larvae to enter 1 seedling was 8 out of 20. During the course of this work, S. whittoni populations were maintained on sweetgum seedlings in clay pots in a greenhouse. Although no records were kept it was apparent that plants placed in sterile soil in which larvae had previously been mixed became more heavily infected than plants inoculated with an aqueous suspension of larvae at the time of planting. More larvae seem to be infective if they have been allowed to become situated in the soil than if they are added to roots in an aqueous suspension. Since all seedlings in the above experiments were inoculated with aqueous suspension of larvae, the rate of infection was never very high.

Reaction to Seedlings and Excised Roots

Steiner (1925) postulated that the amphids of nematodes may be chemoreceptor organs which detect root exudates and enable the worms to find the host plant. There is, however, disagreement among nematologists as to whether nematodes are definitely attracted to roots. The reaction of Meloidogyne hapla (Chitwood, 1949) larvae to tomato seedlings and excised roots has been studied by Wieser (1955). He reported larvae were attracted to seedlings only when the plants were alive and growing. The apical 2 mm of excised root tips were apparently repellent to the worms, while the next 6 mm seemed to be attractive. Widdowson, Doncaster, and Fenwick (1958) found when using seedlings with lateral roots that Heterodera rostochiensis larvae showed preference for certain root

tips around which they congregated. Kühn (1959) conducted experiments with H. rostochiensis larvae to study attraction. He reported that the larvae wander at random. Plants apparently do not attract nematodes chemotropically but only influence the rate of movement of larvae. Weischer (1959), in studying the effect of root diffusates on H. schachtii and H. rostochiensis larvae, concluded the larvae moved faster in the presence of the diffusate. Some of his results suggested attraction but in other experiments no oriented attraction could be observed.

Experiments were conducted to test the reaction of second stage S. whittoni larvae to sweetgum seedlings and excised roots. In the first experiment 1 sterile seedling with roots 2 to 3 cm. long was placed in each of 5 sterile petri dishes containing 2 1/2 per cent water agar. Fourteen-day-old larvae were rinsed 3 times in sterile distilled water and 25 larvae were placed in each dish with a micropipette. The larvae were set approximately 4 cm. from the root. Excess water was removed from around the larvae leaving only a thin film over their bodies. The dishes were kept at room temperature in natural light and observed daily for 1 week. The larvae moved about on the surface and within the agar. They apparently moved at random and there was no indication that they were attracted to the roots. Some larvae moved over and about the roots but were not seen feeding. These larvae invariably moved away from the roots.

This experiment was repeated using larvae of various ages. No attraction to the roots was observed.

In another experiment sterile excised roots 2 to 3 cm. long were

grown on the agar after the method of White (1943). Seeds were planted on sterile nutrient plates containing KH_2PO_4 2.45 g., $Ca(NO_3)_2$ 0.85 g., $MgSO_4$ 3.69 g., Fe citrate trace, and 20 g. per 500 ml of distilled water. Again the larvae seemed to move at random and there was no apparent attraction.

Sweetgum embryos were used in place of excised roots but with the same negative results.

Under the conditions of these experiments second stage S. whittoni larvae apparently moved about randomly. There was no indication of oriented attraction. When on or near the roots larvae did not move more rapidly. Larvae were not observed feeding on roots in these experiments.

Movement in Water Films

The movement of nematodes in water films has been studied by Wallace (1959). He states that, generally, swimming occurs in films thicker than the nematode's diameter and crawling in films much thinner than the nematode's diameter. Wallace conducted experiments to compare the movements of Aphelenchoides ritzema-bosi, a parasite of the leaves of Chrysanthemum, and Heterodera schachtii, a parasite of roots of sugar beet. The habits of these 2 species are very different. A. ritzema-bosi moves about in water films on the outside of the plant above ground level and invades through the leaf stomata, while H. schachtii larvae move in the soil water between soil particles and invade the roots of the host plant.

Wallace found H. schachtii attains greater speed in thin films than

in thick films while A. ritzema-bosi attains its greatest speed in thick films. Both species formed broad tracks in thick films and narrow tracks in thin films. He observed the amount of lateral slip, as indicated by the breadth of the track, was determined by film thickness and maximum speeds occurred when there was no lateral slip. A. ritzema-bosi was the more active of the two species, which accounts for its ability to swim in thick films while H. schachtii did not swim. Both species exhibited the same type of locomotion and attained similar rates of speed in thin films. The ability of A. ritzema-bosi to swim in deep films enables it to move up the stems of plants after rainfall. H. schachtii only encounters thick films when the soil is saturated. Most of the time the soil is at field capacity or drier and this species moves about in the thin film of moisture around the soil particles.

Because S. whittoni is a root parasite, I thought its habits of movement would be more like those of H. schachtii than A. ritzema-bosi. Experiments were conducted to determine whether or not this is the case. Second stage larvae of various ages were observed under 430 x magnification in thick and thin water films on glass slides and on the surface of 2 per cent water agar in petri dishes. A bamboo pick was used to move the worms to the slides and agar surfaces and the thickness of the water film was regulated with a finely drawn-out pipette.

In water films thicker than their body diameter, S. whittoni larvae underwent slow dorsoventral undulations. These movements resembled those of species such as A. ritzema-bosi which can swim, but the larvae made little or no forward progress, generally remaining in one place and

ceasing their undulatory movements after approximately 36 hours. Therefore, S. whittoni larvae did not appear to be able to swim in thick water film. There seemed to be no difference in movement in thick films on glass and agar surfaces. Worms in thick films on agar were never observed entering the agar.

The larvae moved forward readily in water films thinner than their body diameter. They were able to move more rapidly on agar than on glass slides. The route of an individual was easily followed over the agar by the delicate trail formed on the surface of the medium by the worm. The larvae moved faster and for a longer time in instances where the water film was such that little or no lateral slip occurred. Larvae did not move far and soom became inactive when lateral slip was pronounced. As is characteristic of nematode movement, the larvae progressed over the flat surface of the agar by dorsoventral undulatory movements. Thus a lateral surface of the animal was in contact with the substrate. It was easy to ascertain that a nematode was on its side by the conspicuous view of the excretory canal which is best seen in lateral view.

Larvae in thin films frequently entered the agar. Once within the agar the nematodes moved about freely and usually oriented themselves dorsal side up. They frequently remained immobile for several hours but began moving if touched lightly with a probe. If touched on the anterior part of the body the animals, as would be expected, moved posteriorly a short distance and, if touched near the tail, they moved anteriorly. After larvae had been in a dish of agar for several days the agar was riddled with their meandering trails.

The movements of S. whittoni larvae in films of water were more similar to those reported by Wallace (1959) for H. schachtii than A. ritzema-bosi.

Movement Through Sand

An experiment was conducted to determine how far the larvae might move through sand during a 24-hour period. Clean, fine sand was wet with tap water and placed on a suction filter, or allowed to drain, until its moisture content was estimated to be at field capacity. An unsuccessful attempt was made to find a ratio of water to sand volume in which the nematodes would move. The moist sand was packed into a soda straw 210 mm. long and 7 mm. in diameter, the ends of which were sealed with tape. Twenty-five larvae of various ages were picked up in a micropipette. They were allowed to settle toward the nozzle end in a very small volume of water and then injected from the pipette into the straw through a pin hole midway in its length. The amount of water injected with the larvae was small to prevent flooding of the area into which the larvae were introduced. The pin hole was sealed with tape and the straw placed on horizontal supports in a moist chamber. Three straws were prepared in this manner and one was examined each day for 3 days. At the time of examination, the straws were marked off in numbered sections 10 mm. in length to the right and left of the point of entrance of the larvae. The straw was then cut into 10 mm. sections with a razor and the sand within was washed into a dish for examination. The larvae floated free of the sand and could easily be seen under the stereoscopic

microscope.

No larvae were found to have moved farther than the first section, or 10 mm., past the point of introduction even after 72 hours. The maximum number of larvae recovered from any straw was 5. The experiment was repeated with similar results. The explanation for such low recovery is not understood because all larvae were originally enclosed in the straw and it is doubtful that they could have escaped from it. Because of such poor recovery, the fact that larvae were not found more than 10 mm. from the point of entry is not conclusive proof that 10 mm. was the maximum distanced moved. Hannon (1959) conducted a similar experiment using Radopholus similis and Pratylenchus sp. He found that R. similis moved from 48 - 72 mm. in 24 hours. Pratylenchus did not move as far. He, too, had poor recovery of nematodes from the straws.

Tropisms

Geotropism

Clapham (1931) studied tropisms in Dorylaimus saprophilus (Peters, 1930). She observed that D. saprophilus bored into the agar shortly after being placed on its surface. When the culture plate was inverted the worms bored their way back almost to the surface. When she placed the nematodes on a column of agar they bored their way down to the bottom of the dish. In one petri plate all the D. saprophilus moved up the side of the dish and perished from dessication. When placed on 1 inch thick agar the nematodes burrowed a short distance below the surface and moved about freely. Clapham concluded from her results that D.

saprophilus did not exhibit a positive geotropic response. She postulated the nematodes bored into the agar as a result of a stereotropic stimulus.

When second stage S. whittoni were placed on the surface of 2 per cent agar they sometimes bored into the agar. However, most larvae remained on the surface of the agar, seeming to prefer it to the interior of the material. An experiment was conducted using columns of agar. A column of 2 per cent agar was placed on the surface of a 5 mm. thick layer of 2 per cent agar in a petri dish. The column was 5 mm. tall and 4 x 5 mm. wide. Twenty-five second stage S. whittoni larvae of various ages were placed, with a bamboo pick, on top of the column. The dish was kept in the dark at room temperature. The location of the larvae was recorded after 24, 48, and 72 hours. A similar dish was arranged with an agar column 10 mm. tall. The results of this experiment are recorded in Table 5.

TABLE 5

LOCATION OF LARVAE IN RELATION TO TOP OF AGAR COLUMN

No. Hours After Experiment Began	Number of Larvae			
	Top of Column	Sides of Column	Surface of Agar Plate	Within Agar
	5 mm. Column			
24	1	0	23	1
48	3	0	22	0
72	2	0	23	0
	10 mm. Column			
24	3	6	16	0
48	0	2	23	0
72	0	0	25	0

The data in Table 5 indicate that the majority of the larvae apparently showed a positive geotropic response as far as the agar column was concerned. There was little tendency to bore into the agar and go below its surface in the plate. The nematodes did not move down to the surface of the agar plate by boring through the agar columns as D. saprophilus did in some of Clapham's tests. The S. whittoni larvae moved down the sides of the columns in this experiment. There was no indication of a stereotropic response. Most of the nematodes moved about on the surface of the plate after they left the column. Two larvae moved back to the 5 mm. column after 48 hours. This was the only instance of

S. whittoni larvae moving very far in an upward direction. Larvae never moved up the sides of dishes as Clapham noted in D. saprophilus.

Aggregation

Certain nematodes, such as the species of Ditylenchus, show a pronounced tendency to aggregate under adverse environmental conditions. I have seen specimens of an unidentified species of Rhabditis exhibit similar behavior when culture plates began to dry out. Hollis (1958) observed that in mixed populations of nematodes in water Tylenchorhynchus martini consistently formed themselves into aggregations free of other species.

S. whittoni second stage larvae failed to show any indication of gregarious behavior during the course of these experiments.

Heliotropism

Clapham (1931) conducted experiments to determine whether Dorylaimus saprophilus and Rhabditis succaris would react to strong sunlight. Results of tests with D. saprophilus suggested the nematodes showed a negative, heliotropic response. R. succaris gave no indication of a negative heliotropic response but was killed rapidly by sunlight. When attempts were made to cultivate this species in bright light, none of the few eggs produced hatched. Clapham concluded there was no heliotropic response and that certain rays in the light were lethal to the nematodes.

An experiment was tried to determine the response of S. whittoni larvae to bright, indirect sunlight. One-half of a 5 cm. diameter petri dish, containing 2 per cent water agar, was covered with black tape to

exclude as much light as possible from that half. Twenty-five second stage larvae of various ages were placed on the agar on the line of division of these 2 halves. All larvae were placed parallel to the division line. The dish was placed on a laboratory table in bright indirect sunlight. The number of larvae in the light and dark sides of the dish was recorded late each afternoon for 2 days. Thus, larvae were exposed to approximately 2 8-hour periods of bright light interrupted by night.

After the first 8-hour light period, 12 larvae were in the lighted side of the dish and 13 in the dark side. At the end of the second 8-hour light period, 14 larvae were in the lighted side and 11 in the dark side. From the trails on the surface of the agar it was apparent that all larvae had been active.

Under the conditions of this experiment S. whittoni larvae showed no apparent heliotropic response. They moved about the dish as though they were unaffected by bright, indirect sunlight.

Feeding

Plant parasitic nematodes normally feed on 1 plant cell at a time. The worm injects its hollow buccal stylet into the cell and sucks in the protoplasm by the pumping action of the esophagus.

Larvae were best observed feeding on seedlings which had been removed from inoculated sand. The roots were gently washed free of sand and the plants placed in shallow dishes of tap water. Observations were made at 112 x magnification under the stereoscopic microscope.

Larvae were found with the anterior end of the body embedded in the root cortex to a depth of approximately 2 cell layers. Feeding was most commonly observed on primary roots, but it did occur on lateral roots. Feeding was not observed on root hairs. Recently attached larvae fed on cortical cells but deeper penetration of the head apparently took place as the animals matured. Microtome sections of mature females attached to roots revealed that the nematode's head was usually touching the stele. Giant cells were not observed in the root tissue adjacent to the parasite's head.

During larval feeding the median esophageal bulb pulsated with strong, rythmic movements. The valve plates could be seen pulled apart and rejoined again at each beat. No material was seen to enter the esophagi of S. whittoni larvae during the sucking process, nor were any nematode secretions seen to enter host roots. These phenomena might possibly have been seen if observations could have been carried out under higher magnification. The rate of esophageal pulsations in these larvae varied with individuals, ranging from 50 - 60 beats per minute. Rates such as these would not be considered rapid in comparison with those of a species such as Paratylenchus minutus (Linford, Oliveira, and Ishii, 1949). Linford, Oliveira, and Ishii (loc. cit.) recorded rates of 80 to 200 beats per minute in this species.

The esophageal bulb of S. whittoni larvae was active for periods of 1 to 40 minutes followed by rest periods of 1 to 60 minutes. One larvae continued this type of feeding activity for 4 days on a root immersed in water. Most feeding specimens became inactive after the roots to which they were attached were immersed in water for approximately 48 hours.

It was not determined whether the female larvae feed transiently or become permanently attached at 1 locus on the root.

THE FEMALE

In my experiments the second stage female larvae failed to undergo development unless they fed on sweetgum roots. Because of the small number of larvae attached to seedlings in my experiments, some aspects of female development were not worked out. The following paragraphs describe the changes.

Development

Female larvae grew slowly in girth for the first 10 days they were attached to roots. No increase in body length was noted. By the end of 10 days the larvae had increased to approximately 20 μ, or almost twice their original body diameter. In some specimens the biggest increase in size was in the esophageal region, while in others the posterior three-quarters of the body was the most enlarged. By the end of 20 days the nematodes were approximately 4 times their former body width. Molts undoubtedly occurred, but were not observed. Females increased in size until they were somewhat pear-shaped (Fig. 6). By the time the body was this much enlarged the cuticle had become much thickened. Young, spherical, mature females were found on roots after 55 to 60 days. Mature females containing eggs were found after approximately 65 days.

During the course of female development, the head became more deeply embedded in the tissue of the root. As stated previously, recently attached larvae had their heads embedded approximately 2 cell layers deep

Figure 6. Immature female. Head damaged. (Approximately 390 X)

in the roots. The heads of most mature females examined were abutting the stele. The neck region of the nematode was usually short and straight (Fig. 7) but was frequently extended and misshapen (Fig. 8), apparently because of the pressure of the root tissues.

Females of S. whittoni were most commonly found with only the head and neck embedded in the root tissue. The entire bodies of some specimens, however, were completely enclosed within the roots. Females thus enclosed within the root tissues were found by dissecting the roots. In some cases there was a slight swelling of roots in the area immediately around the nematode's body, but usually there was no other external manifestation of the nematode's presence within the root.

Effect on the Host

This nematode species apparently causes little damage to sweetgum. Seedlings with their roots heavily infected were not visibly stunted nor did the roots seem much affected. Some necrotic tissue was visible in areas around female nematodes but it was not very extensive. Neither root-stunting nor excessive branching was noted on parasitized plants. Some roots were slightly swollen in areas around mature females but this was rather uncommon. A study of root tissues around the head of females revealed no evidence of giant cell formation as found in Meloidogyne parasitized roots.

No plants other than sweetgum were inoculated with S. whittoni; therefore, nothing is known of its host range. It is possible that this species may prove to be a detrimental parasite on some plants.

Figure 7. Mature female with vulva located equatorially. The dark central mass is the cluster of ova. (Approximately 390 X)

Figure 8. Mature female with neck embedded in sweetgum root tissue. The vulva is subterminal. (Approximately 390 X)

Egg Sac

Young mature females attached to the exterior of roots were usually covered by a thick, hyaline layer of material (Fig. 9). In older females this matrix usually enclosed eggs. Males were sometimes found coiled within the matrix. Apparently they had copulated with the females and by remaining close by had become enclosed in the matrix. Males were active and probably able to leave the matrix after it had been secreted around them. On young females this material was colorless and had the consistency of gelatin. This structure probably corresponds to the egg sac extruded through the vulva of Meloidogyne females and serves to protect the eggs. The matrix changed in color along with the cuticle of the female as the animal matured and became an egg-packed cyst. The original hyaline appearance of the matrix changed to light tan, golden brown, and dark brown as the animal became older. The matrix adhered to females removed from the roots but could be peeled off with dissecting needles. The matrix from young mature females seemed to be structureless when mounted on slides and examined under oil immersion. Tanned matrix could be peeled off in layers but was optically homogenous. The matrix is apparently secreted in layers by the female.

Pieces of the hyaline matrix from young mature females and tanned matrix from cysts were treated with acid and alkali. There was no apparent difference in the reaction of the new and tanned matrix. The matrix was not visibly affected by 0.2N HCl. It was completely dissolved by concentrated HCl. A solution of 0.2 NaOH partly dissolved the matrix. Pieces of matrix placed in 5 per cent sodium hypochlorite were completely

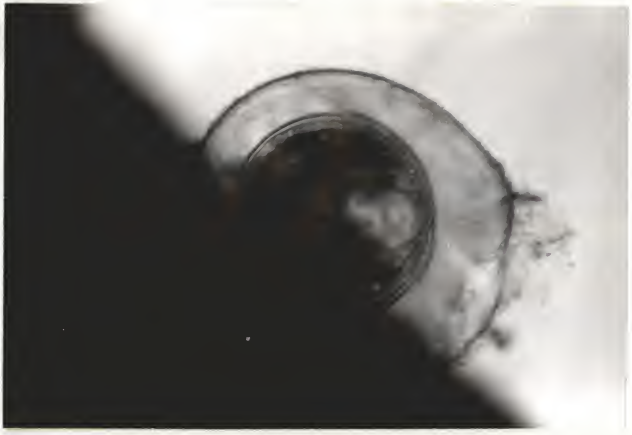

Figure 9. Mature female attached to sweetgum root. Egg sac is surrounding the nematode's body. (Approximately 390 X)

dissolved.

These observations on the egg sac of S. whittoni are similar to those of Bird (1958) on egg sac material from Meloidogyne hapla Chitwood and Meloidogyne javanica (Treub).

Morphology

Most of the specimens used for morphological study were collected from roots of sweetgum seedlings planted in inoculated sand or soil. Females were removed from the roots by teasing the plant tissues apart with needles under the stereoscopic microscope. Care had to be taken to prevent damage to the nematodes embedded in the root tissue.

Very little detail could be seen of the internal anatomy through the thick, dense cuticle. Therefore, both fresh and formalin-fixed specimens were dissected under the stereoscopic microscope to expose the internal organs. Considerable pressure was required from the dissecting needle or scalpel to open the tough cuticle.

External covering

The cuticle is smooth on its outer surface and is not marked by any pattern. It measures from 7 to 10 μ in thickness. The cuticle is white and somewhat translucent in young mature females. When the nematodes grow older the cuticle, or cyst wall, becomes tanned. This also occurs in the genus Heterodera and was studied by Ellenby (1946). He concluded that the tanning process is similar to that which operates in the insect integument. Ellenby (1956) states that the cyst wall of Heterodera rostochiensis is composed of two distinct regions; the outer is fibrous,

the inner, clear. He found that the tanning process begins in the outer region and spreads to the inner zone. When he separated the two zones the process was seen to proceed independently in each zone. No pore canals were observed by Ellenby in the cyst wall.

Bird (1958) studied the cuticle of the female Meloidogyne hapla. He found it to consist of a thin, darkly-staining surface layer covering a homogeneous substance which is divided into 3 layers by 2 darkly-staining bands of material.

The cuticle of adult S. whittoni (Fig. 1E) consists of 2 thick layers separated by a thin transition zone. The outer, dark-staining layer is dense and tough. The next discernable division may be a distinct layer or, more likely, merely a part of the thick, inner layer which stains darker than the rest of the inner layer. Which of these possibilities could not be ascertained for certain. The inner layer is thicker than the outermost layer but does not stain so darkly, and it more readily suffers mechanical damage.

The 2 layers could be separated with dissecting needles, but this was extremely difficult to accomplish. White and tanned cuticles reacted similarly to treatment with reagents.

The results of the reactions of the cuticle with reagents at room temperature are recorded in Table 6.

TABLE 6

REACTIONS OF ADULT FEMALE CUTICLE WITH REAGENTS

Reagent	Visible Reaction of Cuticle	
	Outer Layer	Inner Layer
Boiling Distilled Water (1 hour)	No Change	No Change
Boiling 0.2N NaOH (5 minutes)	No Change	Dissolved
0.2N NaOH (24 hours)	No Change	No Change
5 per cent Sodium Hypochlorite (24 hours)	Dissolved	Dissolved
0.2N HCL (24 hours)	No Change	No Change
Concentrated HCl (24 hours)	Undissolved; turned black	Dissolved

From the data in Table 6 it is apparent that the outer layer of the adult female cuticle is more resistant to these reagents than the inner layer.

Cephalic region

The lip region has a circum-oral elevation. The cuticle is thicker just posterior to this elevation. The cephalic framework is very weak and no details of its structure were ascertained.

Because of the thickness and opaque nature of the cuticle of the adult female, several structures were indistinct or not visible.

Amphidial pores, papillae, cephalids, hemizonid, nerve ring, phasmids, and excretory pore were not observed on females.

Digestive system

The stylet of the female is from 19 to 21 μ long. The tip of the stylet is 54 - 57 per cent of the total stylet length. The knobs are rounded swellings similar to those of the larval stylet.

Details of the esophagus were obscured by the thick cuticle. The procorpus is attached to the knobs of the stylet and is thick and well developed. The median bulb is spherical and very large (approximately 25 μ in diameter) and muscular. The valve within the median bulb is large and heavily cuticularized (Fig. 1B). In most females the median bulb lies within the enlarged part of the body and is thus difficult to see, except in specimens where the neck is long and the bulb lies within the neck region. The structure of the esophagus posterior to the median bulb was not observed. The lumen of the esophagus is heavily cuticularized and prominent. The dorsal gland orifice is from 3 to 4 μ posterior to the stylet knobs.

The structure of the intestine was obscured by the cuticle in whole mounts. The intestine is apparently a delicate structure which may be damaged when the females are embedded in paraffin. A study of serial sections revealed no details of the anatomy of the intestine. When the female becomes packed with eggs the intestine is probably pressed against the body wall and flattened. I have no evidence to confirm this, however.

Reproductive system

The vulva is a small round opening located subterminally in most specimens. A few females were found with the vulva located equatorially (Fig. 7). The vulval lips are flush with the body surface. The short vagina opens into the large, thick-walled uterus. Female S. whittoni are monodelphic, i.e., each has only one ovary. The ovary contains a single row of oocytes. I could not determine whether a spermatheca was present, but it most likely is.

It is not known whether fertilization is necessary in order for reproduction to occur in this species.

THE MALE

Adult males were found in the soil around sweetgum roots and associated with the female egg sac. Although not as active as second stage larvae, the males do move about.

Male second stage larvae molted readily in tap water and their development was easily followed.

When nematodes molt in their natural environment they usually cast off the molted cuticle. However, when specimens are held in a liquid medium they frequently seem unable to shed it. Linford and Oliveira (1940) observed specimens of Rotylenchulus reniformis, which molted in water, with as many as 3 superimposed molts. This phenomenon was also observed in Radopholus similis molting in water by van Weerdt (1959). A firm substratum is apparently required before many nematodes can cast off a molted cuticle.

Second Molt

The larvae became quiescent when approximately 14 to 19 days old. The body was then slightly swollen in the area around the anus where the future cloaca would be located. The swelling was noticeable on both the dorsal and ventral sides of animals seen in lateral view. The genital primordium remained in the 4-cell stage and was 11 μ long by 6 μ wide.

Changes occur in the stylet at this time. The following

observations, made under oil immersion on 1 nematode, were typical. The shaft of the stylet appeared to be less opaque than normal (Fig. 11). The posterior part of the shaft next to the stylet knobs was not as clearly defined as the anterior part joining the stylet tip. The stylet tip and knobs appeared normal. During 8 hours of continuous observation the shaft was seen to slowly dissolve from posterior to anterior. After 5 hours the posterior half of the shaft was no longer visible and the anterior half could still be faintly made out. The shaft had the same appearance as the shaft of individuals placed in HCl. At the end of 8 hours the shaft was no longer visible and the knobs were becoming translucent. During this time the nematode moved its body with occasional jerks toward the ventral side. Twenty-four hours after the last observation, the head of the nematode had begun to be pulled back from the second stage cuticle (Fig. 12). At this time the genital primordium had increased slightly in size by an undetermined number of cells.

Three days later the genital primordium had doubled its length to 22 μ and its width increased to 8 μ. The early development of the gonad indicated the males had but a single testis. The cloacal region was characterized by clear tissue with no globules present (Fig. 13). The head had pulled completely free of the second stage cuticle, the tip of the stylet remaining attached to the old cuticle (Fig. 14). The lining of the amphidial ducts were molted and could be seen attached to the old cuticle. The lining of the terminal excretory duct was molted and was quite long and coiled. Its length could not be measured because of the coils.

Figure 10. Anterior portion of second stage larva. (Approximately 2000 X)

Figure 11. Anterior portion of second stage male larva. The stylet shaft has begun breaking down indicating the beginning of the second molt in the male. (Approximately 2000 X)

Figure 12. Molting second stage male larva. The head is pulled back from the old cuticle to which the stylet tip is attached. (Approximately 2000 X)

Figure 13. Posterior portion of molting male showing differentiation of area in which the spicules and gubernaculum form. Arrow indicates area involved. (Approximately 2000 X)

Figure 14. Molting second stage male larva with head pulled back from the old cuticle. The molted lining of the stoma is a diamond shaped structure around the stylet tip. Arrow indicates molted lining of stoma. Part of the lining of the stylet shaft is a faint strand attached to the posterior end of the stylet tip. (Approximately 2000 X)

Approximately 4 days after the first signs of the second molt the third stage larva became active. The worms were approximately 25 days old. Most of the movement consisted of moving the head backward and forward within the old cuticle. A thin, opaque thread connecting the base of the stylet tip with the head was probably the lining of the dissolved shaft. When the head moved forward and touched the base of the stylet tip this thread folded on itself. When the head moved back away from the stylet tip the thread was pulled straight.

Three and sometimes 4 small round holes appeared in the molted cuticle at this time at about the level of the base of the esophagus (Fig. 15). The cause for these holes in the cuticle is not known. It is at this point in other nematode species that the cuticle frequently breaks open when it is cast off. However, in S. whittoni the cuticle was never observed to be broken open by worms in water.

The tail of the third stage larva was slightly separated from second stage cuticle. The protoplasmic tip remained as part of the molted cuticle. I could not see whether the lining of the anus and phasmids was molted.

The larva became quiescent by the end of the fourth day. The head was extended against the molted cuticle and pushed the stylet tip aside.

Third Molt

The third molt was usually completed about 2 days after the second molt, the larvae being approximately 27 to 29 days old. The fourth stage larva was encased within 2 superimposed molts. The head pulled

Figure 15. Posterior region of esophagus of a molting male. Four small openings occur in the old cuticle opposite the excretory pore. (Approximately 2000 X)

back away from the third molt and moved a little as the third stage larva had done. The linings of the amphidial ducts were molted and attached to the third molt. The lining of the terminal excretory duct was not seen. This third molt was very thin and delicate, and could be seen only at the anterior end. Christie and Cobb (1941) reported the third molt in root knot nematodes to be so thin as to be almost invisible.

The stage of development of the gonad at the time of the third molt varied greatly between individuals. In most cases the genital primordium and the tissue near the cloacal region had grown toward each other until the two groups of cells had almost connected. The spicules and gubernaculum at this time were barely visible. Prior to the fourth molt the tissues composed of cells from the genital primordium and the cloacal region fused to form the complete gonad and the spicules and gubernaculum were completely formed.

Fourth Molt

The fourth molt was usually completed about 2 days after the third molt, or when the nematodes were approximately 29 to 31 days old. The linings of the amphidial ducts were molted and attached to the molted cuticle. The lining of the terminal excretory duct may have been molted but this was not observed. When the head pulled back from the old cuticle the 3 superimposed molts could be seen (Fig. 16). The 3 molts were visible in the tail region also. During certain periods, the body of the male was shortened so that both head and tail were

Figure 16. Molting male with super-imposed second (outer), third (middle), and fourth (inner) molts. Arrows indicate molted cuticles. (Approximately 2000 X)

pulled away from the old cuticles. The male was frequently observed to rotate to right or left approximately 180 degrees within the molted cuticles.

About 1 day after the fourth molt occurred the male extended its body and exerted pressure against the ends of the molted cuticles. The length of the body was greater than the old cuticles. This resulted in the body having slight bends in it (Fig. 17). The nematodes continued moving within the superimposed molts for several days but none ever escaped from the old cuticles.

Males were placed in moist sand to determine whether they could cast off the molted cuticles; however, under these conditions none of the specimens was observed to do so. Males seemed unable to escape from the cuticles unless they molted naturally in soil.

Bird (1959) observed that the hypodermis in the genus Meloidogyne increased in thickness just before molting. He indicates this may point toward the probable role of the hypodermis in cuticle secretion. Measurements were made on the thickness of the hypodermis in molting S. whittoni larvae. No change in the thickness of the hypodermis during the molting process was detected.

Effect of Root Exudate on Molting

Preliminary tests were carried out to determine if molting was affected by leachate from pots planted with sweetgum seedlings. There was no indication that molting of male S. whittoni was affected when the nematodes were held in the leachate. Molting in free-living

Figure 17. Fully developed male with body extended against super-imposed molts. The stylet tip is attached to the outer molt. Arrows indicate areas where super-imposed molts are visible. (Approximately 200 X)

nematodes is probably triggered by intrinsic factors rather than some stimulus from the environment. Rogers and Sommerville (1957) studied molting in parasitic nematodes of cattle. They concluded that the rumen fluid of the host stimulated the nematode larvae to produce an exsheathing fluid which remained active in the absence of the rumen fluid. They postulate the exsheathing fluid comes from cells at the base of the esophagus. These workers believe that adaptations to suspend exsheathment except as a response to extrinsic factors provided by the host may have been important in the development of parasitism in some nematodes.

It is interesting that in the case of S. whittoni females molting is apparently suspended until the proper stimulus, possibly food, is obtained from sweetgum roots. Molting in the males, however, is apparently stimulated by intrinsic factors as in free-living nematodes. The holes appearing in the male cuticle during molting are near the base of the esophagus. Their location is particularly interesting in regard to the theory of Rogers and Sommerville that a molting fluid in nematodes originates in cells at the base of the esophagus and may flow out the excretory pore causing the cuticle to weaken and break in that area. If their theory is correct and if it can be applied to S. whittoni, the 4 holes seen in the male cuticle probably result from the production of some such molting substance near the base of the esophagus, and its excretion onto the cuticle through the excretory pore.

Morphology

Mature males are slightly shorter in length than second stage larvae. Larvae average 426 μ and males, 415 μ.

External covering

The cuticle is much like that of second stage larvae.

Cephalic region

There is no cephalic framework present in the mature male. This structure is lost with the second molt.

Hemizonid

The hemizonid of the male resembles that of the second stage larva but is somewhat larger. It is located just anterior to the excretory pore.

Digestive system

There is no stylet in the male. After the second molt there is an open buccul capsule. The esophagus is ill-defined and probably not functional. The intestine contains numerous globules but as males get older they disappear. Males apparently do not feed.

Reproductive system

The single testis is large, well developed, and filled with small, found sperm. The testis is approximately 7 μ wide and 75 μ long.

The male reproductive system in nematodes is divided into testis, seminal vesicle, and vas deferens (Chitwood and Chitwood, 1950). The

latter two structures could not be distinguished in male S. whittoni.

The spicules averaged 13.2 μ long. They are slender, slightly curved ventrally (Fig. 18), and are not fused at their distal ends. The gubernaculum is small, 5 to 6 μ long, and slightly curved. Males are without a bursa.

Excretory system

The terminal excretory duct is prominent in the male. It resembles the duct of second stage larvae.

Nervous system and sensory organs

The nerve ring was visible on a few specimens and was seen to be almost identical in shape and position to that in the second stage larvae. No amphids or phasmids were observed.

Figure 18. Posterior portion of a male with fully developed spicules and gubernaculum. Two super-imposed molts are visible. (Approximately 2000 X)

PARASITES AND PREDATORS

Protozoan

Numerous second stage S. whittoni larvae removed from soil from the Hatchet Creek area were infected with a sporozoan parasite of the genus Dubosccia. It is not known what species of Dubosccia was parasitizing the larvae. This interesting parasitic protozoan apparently has profound effects upon some soil-inhabiting nematodes.

Thorne (1940) found Pratylenchus penetrans from South Carolina parasitized by a new sporozoan species which he named Dubosccia penetrans. Some workers have questioned Thorne's placement of the organism in the family Nosematidae; however, Hall (1953) agrees with Thorne. He gives the following characterization for the family: "The spores are usually ovoid or pyriform; if more elongated, the ratio of length to thickness is less than 4 : 1. There is only one polar filament." Thorne's description of D. penetrans fits these requirements but he stated that the small size of the spores of D. penetrans made it impossible to see the polar filament.

Thorne found the adult spores of D. penetrans attached to the cuticle of P. pratensis. He observed that the spores draw their sustenance from the nematode's body which results in a breakdown of the tissues. Most of the spores attached to P. pratensis were near the head of tail.

Internal parasitism results when the sporoblast penetrates the cuticle of the nematode and undergoes development within the host. The parasite was particularly destructive to the reproductive system of P. pratensis. However, the entire body might be distended by thousands of spores.

Thorne indicated that D. penetrans may be host specific to P. pratensis because numerous other nematode species found with infected P. pratensis were not parasitized.

Most of the S. whittoni larvae infected with Duboscqia sp. showed the heaviest concentrations of spores around the head and tail, but some spores were attached along the body. Individual nematodes were found with 1 to 14 spores attached to their cuticles. Spores were frequently observed which contained no sporoblast still attached to the cuticle. Presumably the sporoblast had penetrated the cuticle but I never observed the parasites within the larvae.

Larvae infected with Duboscqia sp. were as active as healthy larvae. There was no evidence of damage caused by the sporozoan. However, this may have been due to the fact that the infections were in the early stages.

Attempts were made to infect healthy S. whittoni larvae with Duboscqia sp. by placing them together in soil and in tap water. No healthy larvae became infected under these conditions.

It is obvious that Duboscqia sp. is not confined in its parasitism to P. pratensis. Infected Meloidogyne sp. larvae were found with infected S. whittoni larvae from Hatchet Creek. I have also found this sporozoan parasite on Hoplolaimus sp.

Predaceous Fungus

Duddington (1955) states: "The term 'predaceous fungus' is usually applied to those micro-fungi that capture, kill, and consume microscopic animals." No predaceous fungus was found associated with the various stages in the life cycle of S. whittoni collected from the bank of Hatchet Creek. Therefore, I thought it would be interesting to conduct some experiments to study some of the relationships between a predaceous fungus and S. whittoni.

In the first experiment 3 dishes with Arthrobotrys sp. growing in them were each inoculated with 30 second stage larvae. At the time of inoculation no trapping rings could be seen in the fungal cultures. Duddington (1955) states that most predaceous fungi do not form traps when grown in pure culture, trap formation being initiated when nematodes are added.

After 24 hours hyphae in all 3 plates in the above experiment had numerous sticky rings on them. Most of the larvae had been caught in the rings and were dead. Observation of the cultures under the stereoscopic microscope revealed that most larvae became trapped while moving forward across the agar and entering the loops. Upon being trapped by the fungus in sticky loops worms frequently thrashed about and caused the tail to become caught in nearby loops. The worms ceased struggling about an hour after being trapped. Small outgrowths from the loops grew into the nematode's body, soon filling it with mycelium. After several hours nothing remained of the nematode but the cuticle and stylet.

Predatory Nematodes

Numerous predaceous representatives of 3 genera -- Mononchus, Dorylaimus, and Discolaimus -- were found associated with S. whittoni. Therefore, experiments were conducted to determine the possible role of these predators in the life history of S. whittoni.

The relationships between predaceous nematodes and their nematode prey have interested nematologists for some time. Thorne (1927) studied the effect of certain species of Mononchus on populations of the sugar beet nematode, Heterodera schachtii. He observed the mononchs feeding on males and larvae of H. schachtii. Thorne considered it doubtful that the predators were of any economic importance in control of H. schachtii.

Five adult specimens of each of these genera were placed with a bamboo pick onto the agar of each plate containing the S. whittoni larvae. The plates were observed several hours a day for 7 days. Only Mononchus sp. attacked S. whittoni larvae.

Both predators and larvae moved about the agar freely. Some individuals of both groups moved into and through the agar. None of the predaceous nematodes seemed to be attracted to the larvae. The former readily attacked the latter when their heads contacted the larvae in the process of crawling about the agar. If a larva was placed near (about 1 mm) a predator the latter moved its head about rapidly, sometimes waving it in the air. However, I never observed Mononchus sp. to capture a larva that was placed nearby. Sometimes the predators would move their heads along the entire length of the larva's body and

not attack it. If a larva was placed across the body of Mononchus sp. the predator became greatly agitated and moved about rapidly until its head contacted the larva. After a brief period of searching movements with the head, the Mononchus would crawl away.

The following observations of a Mononchus feeding on a larva were typical of the actions of these voracious little predators. The predators and the larva were seen moving closely at right angles to each other. When the predator's head was midway along the larva's body the former suddenly pressed its mouth tightly against the side of the prey. The larva began thrashing about but its efforts diminished and ceased in a matter of a few seconds. The predator seemed to hold its prey by the combined action of its lips and the suction exerted by the esophagus. The cuticle of the prey was punctured apparently by denticles in the mouth of Mononchus. The esophagus of the predator began strong pulsations and the body contents of the larva were sucked into the gut of the Mononchus.

I did not observe any of the Dorylaimus or Discolaimus feeding on the S. whittoni larvae. Both of these predators were seen to puncture Mononchus with their stylets and draw out the body contents. Mononchus was not seen to prey on Dorylaimus or Discolaimus.

In the natural habitat Cobb (1914) found Mononchus sp. consuming large numbers of the larvae and males of Tylenchulus semipenetrans. Mononchus probably consumes larvae of S. whittoni in the natural habitat. Dorylaimus and Discolaimus, being stylet-bearing predators, may prefer larger prey to S. whittoni larvae and males.

DISCUSSION

An intensive study of the biology of S. whittoni, both observational and experimental, shows that in many respects it is similar to other nematodes that have been investigated by several workers. The greatest degree of similarity is shared with Meloidogyne, Heterodera, R. reniformis, and T. semipenetrans, but S. whittoni deviates in several minor respects which are discussed below.

Embryology

Results of the experiments on the movement of gentian violet into S. whittoni eggs indicate that the vitelline membrane is altered by fat solvents and heat. The egg-shells of normal eggs were stained darkly by gentian violet but neither the vitelline membrane nor the yolk was stained. Eggs treated with fat solvents or heated were completely stained by gentian violet. This indicated that the vitelline membrane had been altered by treatment and the stain entered and colored the vitelline membrane and yolk. These findings are consistent with those of Chitwood and Graham (1940) on the eggs of Strongyloides ratti.

Two polar bodies are frequently seen in the eggs. One is located in the perivitellus space and the second in the extravitellus cavity. The polar body in the latter position is undoubtedly formed before the

vitelline membrane is laid down. The one in the perivitellus space is the last formed. The arrangement of the polar bodies in S. whittoni eggs is similar to that recorded by van Weerdt (1959) for Radopholus similis. However, he frequently observed that the outermost polar body seemed to be in the process of dividing. I did not see this in S. whittoni eggs. The terminal position of polar bodies in S. whittoni eggs thus differs from the position of the polar bodies in Heterodera schachtii and Heterodera avenae. The polar bodies in the eggs of these 2 species are located in the middle of the eggs.

The development of the embryo of S. whittoni up through the 6 cell stage is almost identical with that of Ascaris megalocephala and Turbatrix aceti. Whether this similarity continues throughout subsequent development is not known. I strongly suspect that many similarities do exist throughout the entire development, but I have no facts on which to base this conjecture.

During very early development, cell divisions required 24 hours at room temperature for completion. This is quite slow compared to some free-living forms such as a species of Rhabditis I observed. In this form the early cleavages were completed in 30 minutes or less. The blastomeres could be seen moving during division and the globules within the blastomeres shifted position rapidly during development. However, the difference between the rate of embryological development of these 2 animals is understandable when the length of their life cycles is considered. In the Rhabditis sp. the entire life cycle is completed in 3 days while in S. whittoni approximately 60 days are required.

The stylet in S. whittoni embryos is formed in sections from

anterior to posterior as has been postulated for many stylet-bearing nematodes. The tip is formed about the seventeenth day and the shaft and knobs are formed within the next 3 days.

Hatching of larvae does not seem to require very specific conditions. The chlorine and other additives in tap water did not prevent hatching. Hatching occurs readily in tap water, spring water, distilled water, and rain water. S. whittoni eggs differ in this respect from the eggs of R. similis which van Weerdt (1959) found would not hatch in tap water.

Many cysts removed from the soil contained starved larvae. These larvae had apparently hatched and were unable to escape from the cyst because the vulval opening had become blocked in some manner. Large numbers of the larvae of this species apparently perish in this manner as the larvae seem unable to force their way through the thick cyst wall. Thus it seems that the cyst may often, under certain circumstances, cause the death of the larvae it would normally protect from dessication and predators.

The stylet plays an important role in the normal hatching of the larvae. It is not known whether the stylet actually punctures the eggshell or merely weakens it. It is possible that the eggshell may be weakened or broken down by enzymatic action. It is interesting that although the male larva loses its stylet at the second molt after it has hatched, the male uses its stylet in hatching in the same manner that the female larva does. Thus the stomatostylet of S. whittoni male larvae serves 1 function, that is, in aiding the larva to hatch.

In the female the stylet serves 2 functions. First, it is utilized in hatching, and second, it is used in feeding.

Morphology

Most of the morphological characteristics of second stage S. whittoni larvae are not unique among nematodes. However, I think the results of the experiments on the cuticle are worth recording.

A difference seems to exist in the outer and inner layers of the cuticle of second stage S. whittoni larvae compared to those of Ditylenchus dipsaci, Heterodera glycines males, and Hoplolaimus tylenchiformis adults. From my experiments it appears that the thermolabile membrane of S. whittoni is an inner cuticular layer, while in the other 3 species mentioned above, Chitwood and Hirschmann, working independently, found the thermolabile membrane to be the outermost cuticular layer. The explanation for this difference is not known. The remarks made by Bird (1958) seem pertinent to this situation. He stated: "At our present state of knowledge of the structure of the cuticles of nematodes in general, it does not seem to be wise to attempt to postulate that the presence of an exo- and endo-cuticle is, perhaps, common to all nematodes, as has been done by Wieser; neither is it wise to compare the highly specialized cyst wall of Heterodera with the cuticle of Ascaris lymbricoides. However, attempts such as these to find the common basic pattern of cuticular structure should be of considerable benefit when more is known about these structures in a wider range of nematodes."

My observations on the connection of the cephalids with the longitudinal chords in S. whittoni larvae are consistent with Hirschmann's (1959) findings in Hoplolaimus tylenchiformis and several species of Heterodera. As far as I know, Hirschmann's report and my observations are the only references to date to the connection between the longitudinal chords and cephalids in nematodes.

The molting of male S. whittoni larvae is of particular interest. As has been stated previously, the theory of Rogers and Sommerville (1957) concerning the production near the base of the esophagus of a molting fluid which flows out the excretory pore and breaks down the cuticle, may well apply to S. whittoni. The presence of holes in the cuticle near the excretory pore of molting males provides indirect evidence that such a method may be utilized by this species.

The results of the experiments on the cuticle of the adult female of S. whittoni indicate that its structure is more like that of Heterodera rostochiensis, as reported by Ellenby (1956), than Meloidogyne hapla, as reported by Bird (1958). The cyst wall in both H. rostochiensis and S. whittoni consists of a thick, dense, tough outer layer covering the inner, clear layer. In the adult female cuticle of M. hapla, the outer layer is rather thin and overlays 2 darkly staining bands. The tanning process that occurs in the cuticle of adult female S. whittoni specimens is also quite similar to the tanning in adult females of Heterodera rostochiensis. The cyst wall of S. whittoni probably gives good protection to the enclosed eggs as do the cyst walls of the species of Heterodera.

Physiology

Because the second stage larva is the most active stage in the life cycle of this species, it was the form chosen for physiological experiments. Little if any response could be detected on the part of the rather sluggish males and the sedentary females, but the larvae made good experimental subjects.

Larvae apparently cannot withstand drying. They died almost immediately upon drying on glass slides or in dishes. Quick drying, as well as slow drying, was fatal. Even though the water level in dishes went down gradually in slow drying experiments, the larvae did not appear able to resist the drying. Larvae were not able to withstand drying of the soil in which they were known to be present in large numbers. Thus it appears that the mature cyst and not the second stage larva is the stage most likely to withstand adverse moisture conditions. Although I have no substantiating data, I think the cyst, like that of Heterodera, is no doubt rather resistant to dry conditions. I base this conclusion on the morphological similarities between the cysts of Heterodera spp. and S. whittoni. The cyst of S. whittoni, like that of the species in the genus Heterodera, may serve as a means of dissemination. Cysts could be no doubt transported by wind, water, and animals, but I have no evidence concerning dissemination.

Due to the lack of facilities for temperature studies, a wide range of temperatures could not be tested on S. whittoni. However, of the temperatures used, 24° C was the best suited to the animal's development.

I thought it unlikely that larvae could withstand freezing and experiments confirmed my doubts. However, larvae may possibly withstand lower temperatures when in soil water than in distilled water in dishes. No experiments were conducted to study soil conditions and freezing.

The reactions of larvae to NaCl showed they have little tolerance for concentrations higher than .05N. There is very little information in the literature concerning the tolerance of soil inhabiting nematodes to various salt concentrations. This lack of information is regrettable because these animals live in soil water in which many salts are dissolved, and knowledge of the tolerance would be most valuable in understanding more of their physiological reactions. In experiments with Radopholus similis, van Weerdt (1959) found the nematodes could survive concentrations as high as 2 per cent NaCl but no higher. From this it appears that R. similis, under experimental conditions, can withstand almost 10 times higher NaCl concentration than S. whittoni. However, R. similis is frequently found in rather dry soil situations where the concentrations are probably high compared to the moist habitat of S. whittoni where salt concentrations are no doubt lower. Thus the ability of R. similis to withstand higher salt concentrations may be a result of the forces of natural selection. This, of course, is mere conjecture.

To my knowledge, there are no reports in the literature of experiments in which soil-inhabiting nematodes have been treated with drugs affecting animal nervous systems. Therefore, I conducted experiments in which S. whittoni larvae were treated with several of these.

Although the reactions of S. whittoni larvae to the drugs were predictable, my conclusions are only tentative and must remain so until much further testing is accomplished. Because these tests were conducted using the entire worm, and not an isolated tissue preparation, the behavior of the treated nematodes is probably not a simple response to the drug. Unsuccessful attempts were made to dissect out the various tissues of the larvae with finely drawn-out glass rods and treat them with the above drugs. Because of their small size and delicate structure, the tissues were invariably damaged by this technique so that the experiments failed to reveal any reliable information.

Treatment of larvae with acetylcholine or nicotine sulfate enabled me to study the movements of the stylet under high magnification. The action of the large protractor muscles was easily observed as they forced the stylet outward. No retractor muscles were observed. As far as I know, none has been reported in the literature on stylet-bearing nematodes. Nematologists do not agree as to what causes the stylet to retract, but it has been suggested that the natural elasticity of the cuticle may be responsible. Stylet activity is rarely observed, and when it is, it usually occurs in a specimen feeding on a seedling in culture. Under such conditions study under high magnification is often difficult. The use of acetylcholine and nicotine sulfate may shed light on the mechanism of stylet action.

The fact that larvae in some of these experiments were stimulated to secrete material through the stylet seems significant. This material was presumably a product of the esophageal gland cells whose

ducts open into the lumen of the esophagus. The injection of a material from the esophageal glands of plant parasitic nematodes into host cells is generally believed to occur. Linford (1937a) suggested that such a material probably caused severe damage to host tissues, such as galling of roots by Meloidogyne spp. Experiments by the same author in the same year indicate that these secretions may be enzymatic and function in extracorporeal digestion. In his experiments Linford never observed the injection of such secretions into a plant cell; however, he saw the material flow from the stylets of nematodes removed from the roots and placed in water. He did observe predaceous species of Aphelenchoides injecting material into their nematode prey. The injection of material through the stylet of Helicotylenchus nannus (Steiner, 1945) into a corn root cell was observed by Sledge (1960). Because the secretion of these esophageal gland products has been rarely observed (Linford, 1937a, 1937b, and Sledge, 1960), nematologists have been unable to study them. However, if stylet-bearing nematodes can be stimulated with nicotine sulfate to secrete these materials, as S. whittoni larvae did, it is possible these gland products might be subjects of study.

Staining indicated the presence of adrenalin in the tissues of the larvae. There is apparently no concentration of the material in the region of the nerve ring, but it seems to be spread throughout the tissues. Immersion of the larvae in adrenalin caused characteristic bending of the extreme anterior end of the body. Externally applied adrenalin apparently gained entrance to the body and acted upon the nerves to the somatic musculature causing normal movement of the body.

The explanation for the occurrence of vacuoles in the pseudocoelom of drug-treated worms is not known. I believe because of the rather low concentrations of the drugs used that it is not a result of simple change in osmotic concentration of the environment, but the result of altered metabolism in the animals. This change may have resulted in the production of gaseous products which formed into vacuoles. The vacuoles always disappeared when larvae were placed in tap water and were thus not permanently formed structures. There is also the possibility that materials such as adrenalin may alter cuticular permeability through its effect on metabolism. In that case, waste gaseous products of normal metabolic process may not be able to diffuse out of the animal and thus they collect in vacuoles in the pseudocoelom.

Research on soil nematodes and their reactions to certain drugs may shed some light on the metabolic processes occurring in these animals. Not only would these studies be of considerable academic interest, but they could also furnish information necessary for the chemical control of economically important plant-parasitic nematodes.

Attachment to Host Roots

Results of inoculation experiments show that S. whittoni second stage larvae must be approximately 14 days old before they attach to host roots. The cause for this long period may be that the larvae have to undergo some degree of physiological maturation after hatching before they can, or will, attach to roots and feed. Experiments indicate that attachment is not just a result of time in contact with roots but

is a function of larval age.

No explanation is available for the apparent indifference of S. whittoni larvae to seedlings and excised roots in culture plates. Numerous workers have induced various plant-parasitic nematodes to feed on roots under conditions similar to those used in my experiments.

Life Cycle

In certain aspects the life cycle of S. whittoni resembles that of all 4 genera of sedentary genera reviewed previously. The development of the male of S. whittoni is similar to that of the male of Tylenchulus semipenetrans. However, the male of S. whittoni loses its stylet completely whereas the male of T. semipenetrans retains a small one. As with Rotylenchulus reniformis and T. semipenetrans, only the mature females are known to be parasitic.

Mature female S. whittoni produce a gelatinous-like egg sac in which eggs are laid as in Heterodera, Meloidogyne, Rotylenchulus and Tylenchulus. The shape of the mature female, the structural characteristics of the body wall, and its development into a protective cyst, are more similar to Heterodera than to the other genera.

The feeding habits of S. whittoni and its method of attachment to the exterior of the roots are rather similar to T. penetrans and Heterodera. Although female S. whittoni are sometimes found covered by root tissue as Meloidogyne females are, it is primarily found with most of the body exterior to the root. Thus its mode of attachment nearly approaches that of T. semipenetrans, R. reniformis, and Heterodera.

S. whittoni larvae do not enter root tissue and migrate through it as do larvae of Heterodera and Meloidogyne but the anterior part of the body penetrates at right angles and abuts the stele, as with the larvae of T. semipenetrans and R. reniformis. S. whittoni resembles these 2 species in its effects on host tissue in that it does not cause galling or giant cell formation.

Under the circumstances of my experiments, the life cycle of S. whittoni was completed in approximately 60 days. This is considerably longer than the time required for completion of the life cycle in the other sedentary parasites except T. semipenetrans. Meloidogyne, Heterodera, and R. reniformis require approximately 15 to 30 days for completion of the life cycle but T. semipenetrans requires from 45 to 60 days.

SUMMARY AND CONCLUSIONS

A new species of plant-infesting nematode was found attached to the roots of sweetgum trees (Liquadamber styraciflua). This is described as Sphaeronema whittoni. In addition, studies of embryological development, life history, morphology, and physiology of the various stages of the worm were conducted. References to larvae are to those in the second stage of development. Observations and conclusions are summarized as follows:

1. Hatching of S. whittoni eggs in tap water at room temperature requires approximately 21 days. The first molt occurs within the egg. The male molts 4 times, becoming mature within about 31 days. The mature male, which apparently does not feed, has a degenerate esophagus and no stylet. Females were not observed molting because of difficulties in culturing, but molts very likely do occur. The mature female is a spherical, thick-walled cyst packed with eggs. The entire life cycle of the female requires approximately 60 days.
2. The female, after reaching maturity, is usually attached to the root with its head and neck embedded in the cortex, the head abutting the stele. No giant cells or galls were formed on parasitized roots.

3. Tests with certain drugs were conducted on larvae and gave the following results. When immersed in acetylcholine, they were induced to move the stylet. Nicotine sulfate also stimulated stylet movement in larvae as well as causing them to secrete a substance, possibly saliva, through the hollow stomatostylet. Adrenalin caused peculiar movements and bending of the body.
4. Larvae apparently cannot withstand dessication or freezing.
5. Experiments with various concentrations of NaCl showed that larva cannot tolerate osmotic concentrations higher than 0.05N.
6. Attachment of larvae to roots did not occur in any case until larvae were at least 14 days old. It is not known whether larvae feed transiently or attach permanently at the first feeding site. Larvae did not show that they were attracted to sweetgum roots in culture.
7. Larvae moved best in moisture films thinner than their body diameter. They exhibited a positive geogropic response, but did not react to light either positively or negatively.
8. Larvae exposed to parasitic fungi and to nematode predators were ready prey for these organisms.

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BIOGRAPHICAL SKETCH

Eugene Bondurant Sledge was born on November 4, 1923, in Mobile, Alabama.

After completing the freshman year of college in July, 1943, at Marion Military Institute, Marion, Alabama, he enlisted in the United States Marine Corps. He served with the First Marine Division in two major campaigns in the Pacific Theater of Operations and in the occupation of North China. He was honorably discharged in 1946.

He received the Bachelor of Science degree from the Alabama Polytechnic Institute, Auburn, Alabama, in August, 1949. He was associated with an insurance agency in Mobile, Alabama, from 1949 - 1952. He entered the Botany Department of the Alabama Polytechnic Institute in September, 1952, and received the Master of Science degree in 1955.

He enrolled in the University of Florida in 1956 to pursue graduate studies in Biology. During the course of his graduate studies, he held a position as graduate assistant in the Department of Biology.

He is a member of the following honorary societies: Gamma Sigma Delta, Phi Sigma, and Sigma Xi.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

January 30, 1960

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